Mathematical models of the within-host population dynamics and the evolution of microparasites and immune responses

By: Vitaly V. Ganusov

M.S. Krasnoyarsk State University, 2000

Advisor: Rustom Antia, Ph. D.

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An abstract of a dissertation submitted to the Faculty of the Graduate School of Emory University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Population Biology, Ecology and Evolution Program

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Replication of parasites in vertebrate hosts is counteracted by the immune response, generated for their control. In this dissertation we have investigated the role of immune responses in evolution of microparasites and the maintenance of parasite virulence.

We have developed a theoretical framework for the analysis of the evolution of parasites using models of the within-host population dynamics of microparasites during acute infections in vertebrates. Using this framework we have shown that in contrast with the generally accepted view, higher levels of stochastic host heterogeneity select for higher optimal levels of parasite virulence. We have linked the within-host and between-host dynamics of parasites and demonstrated how the parameters for epidemiological spread of the disease can be estimated from the within-host dynamics. Furthermore, we have shown that changes in the terms describing the rate of parasite transmission from infected hosts and the mechanism of parasite-induced pathogenesis may lead to dramatic differences in the level of virulence to which a parasite evolves.

We have also found that during chronic viral infections, such as infections of humans with HIV, simple models do not allow us to estimate the relative contribution of virus cytopathogenecity (i.e., virulence) and the immune response efficacy in the life-span of virus-infected cells. We nevertheless have demonstrated how the discrimination between two processes can be done with additional experimental data available.

Finally, we have shown that simple models that assume particular mechanisms of cell division and death may give inadequate estimates of cell turnover if the assumptions on the mechanism of cell death are altered. To avoid this problem, we have proposed a novel method for the estimation of turnover of immune cells during and after the immune response to infections that makes very general assumptions regarding the mechanisms of cell division and death. The method can be applied to cell populations for which the number of division a given cell has undergone is known.

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Chapter 1

Evolution of microparasites and maintenance of parasite virulence: a review

Abstract

Why do parasites harm their hosts? Initially, it has been thought that parasites should evolve to become avirulent because survival of the parasite is tightly linked to that of the host. However, for several infections it has been shown that some degree of harm is required for successful spread of the parasite. Subsequently, it has been suggested that if the rate of parasite transmission is correlated with virulence of the parasite, parasites need not evolve to avirulence. Using mathematical models based on this and other assumptions, many further predictions have been generated and a few of these have been tested experimentally. Along with the rapid growth of this adaptive theory of parasite evolution, several nonadaptive explanations for the existence of virulent parasites have also been proposed.

1.1 Introduction

Parasitism is an intimate association between organisms of two or more kinds, one in which, a parasite, obtains benefits from a host which it usually injures (Merriam-Webster dictionary). Although parasites are generally found as the result of degenerative evolution and might be considered as an inferior state of development, parasitism is widespread. While it is difficult to know how many parasites there are in Nature (in part because we do not know the all hosts), it has been estimated that 10-80% of species parasitize on other species to some extend (Windsor, 1998; Poulin and Morand, 2000).

While parasites are ubiquitous, we still do not have good understanding of why parasitism still exists. It has been (and to some extend still is) believed that parasitism is an inferior state and is simply due to recent association between two species (Mims et al., 2001). According to this conventional wisdom, with time parasites should evolve to become avirulent. For several infections, this hypothesis has been rejected on the grounds that virulence is required for parasite transmission, and several alternative hypotheses have been formulated to explain the persistence of parasites.

In this paper, I review three hypotheses on the evolution of microparasites and maintenance of parasite virulence including the conventional wisdom (Section 1.3.1), adaptive theory (Section 1.3.2) and non-adaptive explanations (Section 1.3.3), with the main emphasis on the adaptive theory of parasite evolution. In particular, for these hypotheses I discuss their assumptions, predictions generated from the hypotheses, and their experimental tests. Importantly, in this review I focus only on the evolution of microparasites (or thereafter simply parasites) that are generally defined to include viruses, bacteria, protozoa (unicellular parasites), and fungi. The reader is also referred to several excellent reviews of this field emphasizing both experimental and theoretical results (Levin and Svanborg-Eden, 1990; Bull, 1994; Read, 1994; Frank, 1996; Ebert and Herre, 1996; Levin, 1996; Lipsitch and Moxon, 1997; Ebert, 1999; Read et al., 1999; Schall, 2002; Sabelis and Metz, 2002; Galvani, 2003; Ebert and Bull, 2003). But first, before going into details of

these hypotheses, I will discuss what is virulence and what are the adequate measures of virulence.

1.2 What is virulence?

There have been many discussions on what virulence is in different fields (for example, see recent reviews by Poulin and Combes (1999) and Weiss (2002)). In microbiology, for example, virulence is defined as an ability of the parasite (mainly bacteria and viruses) to replicate in a host (Tortora et al., 1992; Mims et al., 2001). Genes providing the parasite with such an ability are therefore called "virulence factors" (Finlay and Falkow, 1997). In plant biology, parasite virulence is usually but not always associated with an ability of the parasite to infect the host (Read, 1994; Poulin and Combes, 1999; Thrall and Burdon, 2003). While it is hard to find a universally acceptable definition, in the field of the evolution of infectious diseases, virulence is generally defined as *the reduction in host's fitness due to infection with the parasite* (Bull, 1994; Ebert and Herre, 1996).

According to this definition, virulence of a parasite is proportional to the reduction in the number of offsprings, survived to reproductive age, produced by a parasite-infected host in comparison with an uninfected host. In practice, however, this parameter is rarely measured. Instead, other indirect alternative measures of the reduction of host fitness due to infection have been applied. Among the most widely used are the following: (i) the case mortality (a probability that an infected host will die following infection), (ii) the parasite-induced host mortality rate (the rate at which hosts in the population die due to infection with the parasite), (iii) the average life-span of infected hosts, (iv) the lethal dose 50, LD_{50} (an initial dose of the parasite required to kill 50% of infected hosts during the infection), and (v) the within-host parasite's growth rate r. While case mortality and host mortality rate are more direct measures of virulence, LD_{50} and r are clearly not.

Given many alternative measures to choose from, optimally, the virulence measure used should represent the reduction in host fitness due to the infection as close as possi-

ble. Sometimes, however, it is not achieved, and for some infections, conventionally used measures of virulence may be truly inadequate.

For example, many parasites that achieve high densities within their hosts cease host reproduction (Baudoin, 1975). If infection occurs early in life and is life-long, such castration represents a large reduction in host's fitness. On the other hand, since infected hosts generally do not die following infection and in some cases may survive longer because more resources are devoted to the growth (Baudoin, 1975; Ballabeni, 1995), such parasites are relatively avirulent if virulence is measured by the case mortality or host mortality rate (virulence measures employed in most mathematical models of parasite evolution).

In some cases, it might even be impossible to compare different infections by their virulence using alternative virulence measures. For example, which infection is more virulent for humans, smallpox or HIV? Smallpox can have case mortality up to 40% (Behbehani, 1983; Berche, 2001) while HIV is nearly 100% lethal (Buchbinder et al., 1994). On the other hand, the duration of infection with smallpox until the host's death or recovery is less than one month while in HIV infection it takes on average 10-12 years for infected hosts to die if untreated (Longini et al., 1989; Longini, 1990; Buchbinder et al., 1994; Mellors et al., 1996). This can be translated into the host mortality rate for smallpox $\alpha = 0.4/30 \approx 1.3 \cdot 10^{-2}\ day^{-1}$ and HIV $\alpha = 1/(10 \cdot 365) \approx 2.7 \cdot 10^{-4}\ day^{-1}$. Obviously, two measures of virulence (case mortality and host mortality rate) rank these two infections differently. But which of these two measures is more appropriate to estimate virulence of each infection?

Since acute infections by definition are infections of short duration, it is likely that the parasite causing the infection will reduce host's reproductive success only if the host does not survive the infection. Therefore, if infection occurs early in life and does not impose any long-term consequences on subsequent survival and reproduction of hosts survived the infection, the most appropriate measure for virulence of parasites causing acute infections (such as smallpox) is the *case mortality*. In contrast, chronic infections often last for the life-span of an infected individual. If infected hosts reproduce less than uninfected hosts,

then the *host mortality rate* due to infection, that is $\frac{\text{case mortality}}{\text{duration of infection}}$, is the appropriate measure for virulence of parasites causing chronic, persistent infections (such as HIV). Thus, smallpox and HIV cannot be compared by their virulence if one uses alternative measures of virulence. However, the infections can be compared if one estimates the true reduction of host fitness due to these infections, the task clearly more difficult than estimation of case mortality or host mortality rate.

1.3 Why are parasites virulent?

In this section, I review three hypotheses for the evolution of parasite virulence including the conventional wisdom hypothesis (Section 1.3.1) and adaptive (Section 1.3.2) and non-adaptive (Section 1.3.3) explanations.

1.3.1 "You shall not murder": a conventional wisdom

> "Given enough time, a state of peaceful coexistence eventually becomes established between any host and any parasite... Throughout nature, infection without disease is the rule rather than the exception." (Dubos, 1965, p. 190)

> "It is a conflict between man and his parasites which, in a constant environment, would tend to result in a virtual equilibrium, a climax state, in which both species would survive indefinitely " (Burnet and White, 1972, p. 20-21)

> "In general terms where two organisms have developed a host-parasite relationship, the survival of the parasite species is best served, not by destruction of the host, but by the development of a balanced condition in which sufficient of the substance of the host is consumed to allow the parasite's growth and multiplication, but not sufficient to kill the host." (Burnet and White, 1972, p. 29)

> "... from an evolutionary point of view, successful microbes must avoid extinction, persist in the world, multiply, and leave descendants." (Mims et al., 2001, p. 3)

Parasites require their hosts for replication and transmission and death of the host often means death for the parasite. A conventional wisdom suggests that parasites should evolve to reduce the damage done to the host and eventually to become avirulent. This pacifistic view on parasite evolution became widely spread in part because of influential books by Dubos (1965) and Burnet & White (1972).

There are two major observations that are used in support of this hypothesis. First, many parasites do not cause severe disease in their natural hosts, i.e., in hosts where long coevolutionary history of the parasite and its host is known or suspected. For example, myxoma virus induces a very mild disease when infects its natural host, the American rabbit (Fenner and Ratcliffe, 1965). Simian Immunodeficiency virus (SIV) infection of natural hosts such as SIVsm infection of sooty mangabeys or SIVagm infection of green monkeys does not lead to immunodeficiency despite rapid replication of the virus and high rates of T-cell turnover (Rey-Cuille et al., 1998; Chakrabarti et al., 2000; Diop et al., 2000). Many Orthomyxo-, Arena-, and Hantaviruses cause asymptomatic infection in their natural hosts (influenza A in birds, arena and hantaviruses in rodents) (Murphy and Webster, 1996; Peters et al., 1996; Lednicky, 2003). Causing little pathology and being successfully transmitted are therefore ideal traits of the parasite.

Second, some parasites are extremely virulent when they encounter a novel host[1]. With time, parasite virulence (generally associated with the severity and incidence of the parasite-caused disease) declined. Several infections that have been brought into the New World by Europeans such as smallpox, measles, and influenza appear to follow this pattern. Initial severity of these infections in Indian populations is well known (Dubos,

[1]It should be emphasized, however, that these examples are rather exceptions than the rule since many such encounters most likely occur unnoticed due to inability of the parasite to replicate in a new host (Ebert, 1998).

1965; Burnet and White, 1972; Mims et al., 2001). The reasons for such initial severity and its decline with time, however, are still debated (Black, 1992). Both these observations have been interpreted that parasites evolve to cause less harm to their hosts.

It is important to emphasize that the decrease in parasite virulence has not been directly measured in these examples. The only well documented change in virulence of a parasite after introduction into a new population is the evolution of myxoma virus in Australian populations of European rabbits (reviewed in (Fenner and Ratcliffe, 1965; Fenner and Fantini, 1999)). The initially introduced myxoma virus strain was very lethal to wild and laboratory rabbits (with case mortality $> 99\%$). In several years following the introduction, the average virulence level of the virus as measured in laboratory rabbits has declined (see Figure 1.1), in accord with the prediction of the conventional wisdom hypothesis. Later, however, rabbits became more resistant and that in turn led to the selection of more virulent strains of the virus that currently kills approximately 50% of wild and more than 99% of laboratory rabbits (Fenner and Fantini, 1999; Merchant et al., 2003b; Kerr et al., 2003; Merchant et al., 2003a, see Figure 1.1).

It is generally interpreted that conventional wisdom assumes that parasites should evolve to avirulence. While this might be generally correct, some examples on the evolution of infectious diseases given, for instance, by Dubos (1965) imply that host evolution may be an important factor and that changes in host resistance might be responsible for lowering virulence of human parasites (Dubos, 1965). One example where most likely host evolution towards resistance resulted in a relative benign infection is myxomatosis. The myxoma virus causes very mild disease in its natural host, American rabbits *Sylvilagus brasiliensis* and *S. bachmani*, and initially caused severe disease in European rabbits in Australia (Fenner and Ratcliffe, 1965; Fenner and Fantini, 1999). Although there have been changes in virulence of the virus since its first introduction in Australian rabbit populations as well as in the resistance of rabbits, the virus is still quite virulent. Immunological studies of myxoma pathogenesis in resistant and susceptible rabbits suggest that it is the host immune response that limits the spread of the virus in resistant rabbits, but causes

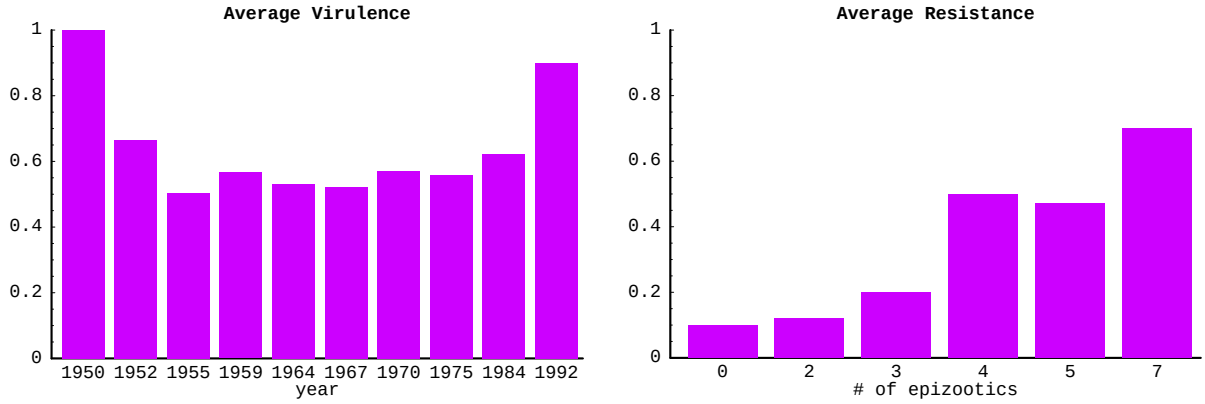

Figure 1.1: Changes in the level of average virulence of the myxoma virus and average resistance of rabbits after introduction of the virus in rabbit populations in Australia. Left panel shows the changes in average virulence. Average virulence was calculated using the prevalence data on the different strains of the virus and assigning arbitrary value 1 to the most virulent strain (grade I) and 1/5 to the least virulent strain (grade V). Data are taken from Fenner and Fantini (1999). Right panel shows the changes in average resistance after several epidemics at the lake Urana in Australia. Average resistance was calculated as a fraction of survived hosts after challenge of nonimmune rabbits with the virus of grade III virulence. Data are from Fenner (1983).

pathology in susceptible hosts (Best and Kerr, 2000; Kerr and McFadden, 2002). Similarly, SIVsm/HIV-2 is non-pathogenic in its natural host, sooty mangabeys, yet causes AIDS in infected humans (Peeters et al., 1991; Rey-Cuille et al., 1998; Chakrabarti et al., 2000). Thus, host evolution rather than parasite evolution may be the reason why some infections are mild.

One of the shortcomings of the conventional wisdom hypothesis is that it is hard to test it. For some infections, however, this hypothesis has been rejected on the grounds that some virulence was required for the infection to be transmitted. For example, water diarrhea caused by *V. cholera* appears to enhance the transmission rate of the bacteria. Some other parasites are transmitted only from dead hosts and thus killing the host may be advantageous for such parasites (Lafferty, 1999; Ebert and Weisser, 1997; Ebert et al.,

2000).

While some infections of humans, such as smallpox and TB, have been in the human population for thousands of years, it still could be argued that not enough time has passed to select for strains with reduced virulence. This argument of "insufficient time" that could be applied to many virulent infections is not easy to reject because we don't have good estimates of how long it should take for a parasite to become avirulent. From the serial passage experiments (SPEs) we know that parasites can evolve quite rapidly (reviewed in Ebert (1998)). However, it is not clear if rates of parasite evolution observed in SPEs are close to natural since in SPEs very high numbers of parasites are transferred in a non-natural way such as using syringe needles. On the other hand, a rapid change in average virulence of the myxoma virus after the initial introduction (Fenner and Ratcliffe, 1965) and a rapid decline in prevalence of toxin-producing bacteria (*C. diphtheriae* and *B. pertussis*) following introduction of anti-toxin vaccines (Taranger et al., 2001; Soubeyrand and Plotkin, 2002) does suggest that parasites may evolve their virulence in a matter of years.

This is at least one area in which theory could provide some answers. It is often emphasized that parasites may evolve more quickly than their hosts because of their large population sizes and short generation times (Mims et al., 2001). However, new infections are generally initiated by only a few parasites (Sacristan et al., 2003), and therefore, the effective population size of parasites may be much less than the size reached by the parasite in a given infected host. How this and other factors may affect the rate of parasite evolution has not yet been addressed (De Leo and Dobson, 2002).

1.3.2 "Enlightened theory": the adaptive theory of parasite evolution

> "Although successful parasites cannot afford to become too pathogenic, some degree of tissue damage may be necessary for the effective shedding of microor-

> ganisms to the exterior, as for instance in the flow of infected fluids form the nose in the common cold or from the alimentary canal in infectious diarrhoea." (Mims et al., 2001, p. 5)

The conventional wisdom has failed to realize that parasites evolve to maximize their reproductive success and not the duration of infection or the probability of species survival. In some cases reproductive success of a parasite can be calculated as the number of newly infected hosts during the infection. Although a large duration of infection is beneficial to the parasite, having a shorter infection may be still advantageous if shorter duration is compensated by an increase in the infection rate of new hosts. Therefore, this trade-off between the rate of infection and the duration of infection will determine the optimal parasite infectivity and duration of infection if parasites evolve in the presence of such a trade-off. A shorter duration of infection generally is associated with higher virulence of the parasite measured by the host mortality rate. The suggestion that parasites evolve in the presence of such a trade-off (or additional trade-offs) is the backbone of the adaptive theory of parasite evolution. There has been a number of experimental studies aimed to examine the assumptions of the adaptive theory and theoretical studies aimed to make further predictions on the evolution of parasites assuming that the trade-offs are present.

Anderson and May (1982) in their influential paper proposed a theoretical framework for the analysis of the evolution of parasites. For the epidemiological spread of the infection caused by a parasite, they considered the basic reproductive number of the parasite, R_0, that is, the average number of new infections caused by an infected host introduced into a wholly susceptible population. For directly transmitted infections R_0 is:

$$R_0 = \frac{\beta N}{d + \alpha + \nu}, \tag{1.1}$$

where β is the rate of parasite transmission from infected hosts[2] and α, d and ν are the rate constants for the parasite-induced and natural host mortality and recovery, respectively,

[2]and simultaneously, the rate of infection of susceptible hosts.

and N is the density of susceptible hosts. In this model, the parasite-induced host mortality rate α is taken as a measure of virulence.

If an infected host can be occupied only by one parasite strain, then the parasite with the maximal R_0 will exclude others from the population (Anderson and May, 1982; Bremermann and Thieme, 1989). If β, α, and ν are all independent, then selection will favor parasites that are highly infectious ($\beta \to \infty$), avirulent ($\alpha \to 0$), and causing persistent infections with no recovery ($\nu \to 0$). Based on experimental observations Anderson and May proposed that these parameters at least for some infections may not be independent. For example, transmissibility and host recovery rate may depend on the parasite-induced host mortality rate, $\beta = \beta(\alpha)$ and $\nu = \nu(\alpha)$. Such dependencies are often called trade-offs even though the correlation $\beta = \beta(\alpha)$ when it exists is generally positive. For some appropriately chosen functions $\beta(\alpha)$ and $\nu(\alpha)$, the maximum of R_0 is achieved at intermediate levels of α.

Anderson and May used this theory to predict the optimal level of virulence of the myxoma virus evolving in populations of European rabbits in Australia after its initial introduction in 1950 (Figure 1.1). After the introduction of a very virulent virus strain, virus virulence declined dramatically for the following several years and was stably maintained for some time at intermediate levels. From laboratory experiments it became clear why virus strains with low and high virulence had lower fitness than strains with intermediate virulence: strains with low virulence caused only mild diseases in rabbits which survived for long periods of time. However, the probability of virus transmission from such rabbits was very small because of small densities of the virus in the skin lesions of infected hosts. Thus, even at long duration of infection, these strains do not obtain high total transmission. Similarly, highly virulent strains, were transmitted more efficiently but only for a very short time, obtaining low total transmission (Fenner and Ratcliffe, 1965).

Using the data on the case mortality ($M = \alpha/(\alpha + \nu)$) and the average duration of infection in hosts that died following infection ($\Delta \approx \alpha^{-1}$) caused by different viral strains (grades I-V), Anderson and May estimated the trade-off between the host recovery rate

ν and the host mortality rate α (Anderson and May, 1982; May and Anderson, 1983, Figure 1.2). Using this trade-off and assuming that transmissibility β does not depend on virulence α, they found the optimal virulence level, at which R_0 is maximal (Figure 1.2). The obtained value, $\alpha^*_{theory} \approx 0.013\ day^{-1}$, was close to the observed $\alpha^*_{observ} \approx 0.041\ day^{-1}$ (Fenner and Ratcliffe, 1965; Anderson and May, 1982). This prediction was then improved by assuming a positive correlation between the probability of parasite transmission from infected to uninfected hosts for two vectors, fleas and mosquitoes (Massad, 1987; Dwyer et al., 1990, Figure 1.2). Thus, the trade-offs between parasite transmissibility, host recovery rate and parasite-induced host mortality rate determine the level at which parasite fitness is maximal.

Some of these trade-offs seem to be intuitively obvious. For example, higher average parasite load in an infected host may generally lead to higher transmissibility and higher virulence, thus leading to a positive correlation between the two traits. For some infections such correlation has indeed been found (see Table 1.1 for some examples) but not for others (Davies et al., 2001).

Two points need to be emphasized. First, many infection of practical interest have not been rigorously tested as to whether there are trade-offs that may constrain their evolution. Such studies should establish that (1) there is a variation in the parasite population in the degree of virulence, and (2) different parasites strains have different fitnesses (for example, total transmissions) upon which natural selection can act. Despite the absence of such knowledge, the trade-off hypothesis has been widely used to explain changes in virulence of many distinct parasites without actual demonstration that the trade-offs for such infections exist (Ewald, 1994; Dieckmann et al., 2002).

Second, in most of the studies where trade-offs have been established, the causes of these trade-offs are generally not well understood. It is likely that the causes of the trade-offs can be simple or complex for different host-parasite associations.

For example, the correlation between transmissibility and virulence may be simply because of the direct linkage between these two traits. For a horizontally transmitted mi-

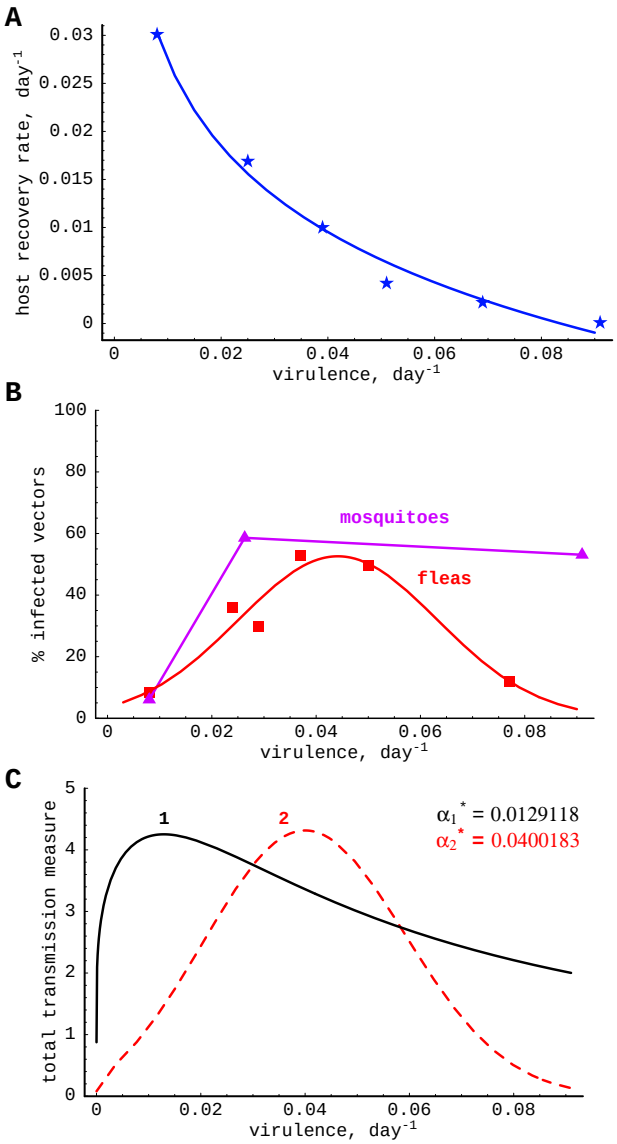

Figure 1.2: Measured trade-offs for the naturally occurring strains of the myxoma virus. Panel A shows the trade-off between host recovery rate ν and parasite-induced host mortality rate (virulence) α. Panel B shows the trade-off between the probability of parasite transmission from an infected to susceptible host for two vectors, mosquitoes ($\triangle$) and fleas ($\square$). Data are taken from Fenner et al. (1956), Mead-Briggs and Vaughan (1975), and Anderson and May (1982). Panel C shows the changes in the basic reproductive number R_0 calculated when only the trade-off between ν and α is known (1), or when both trade-offs are used (2). To calculate R_0 the following approximations for the trade-offs were used: $\nu = -0.032 - 0.013\log(\alpha)$ (Anderson and May, 1982), $\beta = \exp\left[-\frac{(\alpha-0.044)^2}{0.0007}\right]$ (fleas).

infection	**transmission**	**virulence measure**	**references**
myxoma virus in rabbits	mosquitoes, fleas	case mortality	(Fenner et al., 1956; Mead-Briggs and Vaughan, 1975)
influenza A in mice	direct (airborne)	pathology of lungs	(Schulman, 1967, 1970)
malaria (*P. chabaudi*) in mice	mosquitoes/needle	anemia/body wasting	(Mackinnon and Read, 1999)
G. intestinalis in Daphnia	fecal-oral	host survival time	(Ebert, 1994; Ebert and Mangin, 1997)
phage f1 in *E.coli*	environment	host generation time	(Messenger et al., 1999)
CM virus in tomato plants	direct/vector-borne	general pathology	(Escrui et al., 2003)

Table 1.1: Examples of infections for which the correlation between the transmission success of a parasite and parasite virulence has been established.

crosporidian parasite *Glugoides intestinalis* of the water flea *Daphnia magna* the following relationships have been established: the number of parasite spores per host is inversely correlated with the life-span of infected hosts (i.e., the duration of infection) but is positively correlated with the amount of parasites expelled into the environment. A higher spore density in the environment, on the other hand, leads to a higher probability of infection of uninfected hosts (Ebert, 1994; Mangin et al., 1995; Ebert and Mangin, 1997). Thus, in this case, the correlation between virulence ([life-span of infected hosts]$^{-1}$) and transmissibility is simply mechanical: an increase in the parasite production rate may shorten the infection, but simultaneously leads to an increase in the probability of infection of a new host.

One the other hand, although measured, the underlying mechanisms of the correlation between transmissibility and virulence for the malaria parasite of rodents, *Plasmodium chabaudi*, are less clear because of the more complex nature of the infection (Mackinnon and Read, 1999). In this infection virulence, often measured by host morbidity, weight loss and case mortality following the infection, is caused by the replicating asexual stage of the parasite (merozoites) mainly by depletion of red blood cells. Transmission to the mosquito vector is due to a terminally differentiated sexual stage (gametocytes). Because of the complexity of the differentiation pathway of the asexual to sexual stage in *Plasmodium sp.* (Taylor and Read, 1997; Dyer and Day, 2000), the underlying mechanisms of the correlation between transmissibility and virulence are not well understood.

Importantly, for parasite strains recovered from host populations in Nature, a correlation between transmissibility and virulence may be observed even without a physical linkage between these two traits. Such a correlation may simply arise because more virulent parasite strains are evolutionary selected for higher transmissibility. For example, theoretical studies on the evolution of parasites in spatially heterogeneous environments suggest that for a given level of virulence (a probability of host dying following infection) parasites with too low and too high rates of transmission will not be maintained in the host population (Haraguchi and Sasaki, 2000; Rauch et al., 2003). This is because parasites

with too low transmissibility on average kill their hosts before they are transmitted while parasites with high transmissibility infect and kill locally all susceptible hosts and are not able to be transmitted to next cluster of hosts (Haraguchi and Sasaki, 2000; Rauch et al., 2003).

Despite these shortcomings, the adaptive theory forms the basis for the analysis of the evolution of infectious diseases. Its great advantage is that it relies on the trade-offs to predict evolution of parasites, and therefore, can be rejected if the trade-offs for the parasite-induced disease are not observed.

Further specific predictions of the adaptive theory, their tests and critique

Since Anderson and May, many theoretical studies have made specific predictions on how parasites should evolve in different conditions (for a relatively recent theoretical review see Frank (1996)). However, only a few of such predictions have been tested experimentally (see, for example, an excellent discussion by Schall (2002) for the testing of different predictions of the adaptive theory applied to malaria parasites of lizards).

1.3.2.1 Natural host mortality rate

Many models of parasite evolution predict that an increase in the parasite-independent host mortality rate should lead to selection of parasites that kill their hosts more rapidly and therefore are more virulent (Frank, 1996; Ebert and Mangin, 1997; Day, 2002b). This is simply because a shorter life-span of the host often leads to a shorter duration of infection that in turn selects for more rapidly replicating parasites. This can also be seen by maximizing the basic reproductive number R_0 given in eqn. (1.1) with respect to α assuming a positive correlation $\beta = \beta(\alpha)$. The optimal virulence level obtained at $\beta = \beta_0\alpha/(\alpha + c)$ and given by eqn. (1.4) increases with increasing natural host mortality rate d. Similarly, a longer host life-span would lead to selection of slower replicating

parasites with lower virulence. For several experimental systems these predictions have been tested with variable success (Ebert and Mangin, 1997; Ebert, 1998; Elena, 2001; Cooper et al., 2002).

In serial passage experiments (SPEs) where parasites are manually transmitted from an infected to a new host, parasites evolve higher within-host growth rate and concurrently virulence if the transmission event occurred early in infection (reviewed in Ebert (1998)). Since early transmission mimics high host mortality rate, this observation is consistent with the prediction of the adaptive theory. Similarly, parasites that are serially passaged late during the infection evolve lower growth rate and virulence (Dobson and Owen, 1977; Elena, 2001; Cooper et al., 2002).

While consistent with the theory, these results cannot be directly applied since transmission in SPEs is generally done manually at one fixed time point and therefore does not correspond to the natural way of parasite transmission. Ebert and Mangin (1997) attempted to test the above prediction using a quasi-natural setting. They allowed a microsporidian parasite (*Glugoides intestinalis*) to evolve in populations of *Daphnia magna* while applying two regimes with high and low natural host mortality. High natural host mortality was achieved by transferring only 10-20% of hosts into a new aquarium, while the control (low host mortality) was left unmanipulated. Surprisingly, parasites in the high mortality rate regime evolved lower growth rate and virulence (measured as the life-span of infected hosts) than those from the low mortality regime. The authors speculated that in unmanipulated populations a longer exposure to the parasites in the water led to an increased frequency of hosts infected with several distinct strains of the parasite (i.e., multiply infected hosts). This in turn selected for more rapidly growing parasite strains (Ebert and Mangin, 1997, Section 1.3.2.4). Although direct evidence for the occurrence of multiple infections in this experiment was lacking, theoretical analysis does suggest that when multiple infections are allowed, higher virulence is expected to evolve at low natural host mortality rate (Gandon et al., 2001a). This is simply because the longer duration of infection increases the probability of an already infected host to be super-infected with a

more virulent parasite strain.

Other particular "details" of the infection may also change the prediction of how natural host mortality affects the optimal level of parasite virulence. For example, if the duration of infection is much shorter than the average host life-span, then reduction in the host life-span should not dramatically affect the optimal level of parasite virulence. Furthermore, theory suggests that higher host mortality can select for lower virulence depending on whether there is an interaction between parasite-induced and natural host mortality rates and on how virulence is measured (Williams and Day, 2001; Day, 2002b; Choo et al., 2003). These examples clearly illustrate that particular details of a given parasite-host association are important in predicting the evolution and the optimal level of parasite virulence even if the basic assumptions of the adaptive theory (such as the trade-offs outlined above) are fulfilled.

1.3.2.2 Host recovery rate/host resistance

It appears that it is difficult to make general predictions on how host resistance would affect the evolution of parasites because of many ways the resistance can be provided. In plants and invertebrates resistance is often defined as inability of the parasite to infect the host (Thrall and Burdon, 2003; Rolff and Siva-Jothy, 2003). In contrast, in vertebrates, host resistance is often associated with the host ability to mount an effective immune response to quickly clear the infection.

General theory predicts that an increase in the recovery rate should select for more virulent parasites (Frank, 1996; Antia and Lipsitch, 1997; van Baalen, 1998; Gilchrist and Sasaki, 2002; Day and Burns, 2003; Andre et al., 2003). This is, similar to the previous case, because a higher recovery rate leads to a shorter duration of infection forcing parasites to evolve higher growth rate and virulence (see also eqn.(1.4)).

In contrast, increased host resistance to the infection (which can be achieved by both higher recovery rate and resistance to initial infection) may select for higher or lower virulence level depending on particular mechanisms of resistance (Gandon and Michalakis,

2000). As far as I know there have been no studies where these predictions have been rigorously tested. However, an increase in virulence of the myxoma virus following an increase in resistance of rabbits to the infection in the past few decades in Australia is consistent with the theoretical prediction (Fenner and Fantini, 1999, Figure 1.1).

Gandon et al. (2001) have proposed that imperfect vaccines that, on the one hand, increase host resistance, but, on the other hand, allow replication and transmission of parasites, may lead to evolution of parasites with lower or higher virulence depending on the type of the vaccine. Vaccines blocking new infections or transmission from infected hosts are predicted to select for lower virulence because they would reduce the intensity of superinfection of already infected hosts, and therefore the frequency of multiply infected hosts. This is turn should select for lower virulence (Section 1.3.2.4). In contrast, vaccines that reduce the within-host replication rate of parasites or their virulence are expected to evolve higher virulence in the presence of vaccinated hosts. This is because such vaccines remove the cost of virulence (Gandon et al., 2001b, 2003).

The assumptions of the mathematical model and generality of its predictions have been heavily criticized (Smith, 2002; Ebert and Bull, 2003; Andre et al., 2003). This was in part because the theoretical prediction that vaccination against toxins (i.e., virulence) should select for more virulent parasite strains was in contrast with the observed reduction in prevalence of toxin-producing *Corynebacterium diphtheriae* and *Bordetella pertussis* after introduction of anti-toxin vaccines (Soubeyrand and Plotkin, 2002). Toxin production bears a cost and in the presence of anti-toxin immunity, bacteria not producing toxins have selective advantage. Although *ad hoc* changes in the model to include the toxin cost led to "improved" predictions, this example again emphasizes the role of particular details in predicting the evolution of parasites.

1.3.2.3 Epidemic vs. endemic diseases (early vs. late transmission)

The adaptive theory assumes that parasites evolve to maximize their reproductive success. From theoretical studies it became clear that for epidemic and endemic infections the

reproductive success of parasites might be calculated in different ways (Frank, 1996). For epidemic infections the number of susceptible hosts is large and parasite strains that infect the hosts more rapidly will have selective advantage (Knolle, 1989; Lenski and May, 1994). Thus, in epidemic infections parasites will maximize the net growth rate of the number of infected hosts, that for directly transmitted diseases in the absence of the within-host competition is simply:

$$r = \beta N - (\alpha + d + \nu), \tag{1.2}$$

where the parameters are the same as in eq. (1.1). In contrast, for endemic diseases there is always dearth of susceptible hosts, so parasites that infect the maximum number of hosts during the infection, will have selective advantage. Thus, in endemic infections at some conditions[3], parasites will maximize their basic reproductive number R_0 that for directly transmitted diseases is given by eq. (1.1) (Anderson and May, 1982; Bremermann and Thieme, 1989; Frank, 1996).

If there are (appropriate) trade-offs between parasite characteristics both fitness measures are maximized at intermediate values of virulence. For example, if $\beta = \beta_0\alpha/(\alpha + c)$ and $\nu = \text{const}$, the optimal virulence for two infection types are:

$$\alpha^*_{epidem} = \sqrt{cN\beta_0} - c, \tag{1.3}$$

$$\alpha^*_{endem} = \sqrt{c(d + \nu)}. \tag{1.4}$$

where $\alpha^*_{epidem} > \alpha^*_{endem}$ for any parameter combination. It can be also shown (see Appendix) that $\alpha^*_{epidem} > \alpha^*_{endem}$ for any trade-off $\beta = \beta(\alpha)$ if the host recovery rate depends weakly on virulence. Importantly, using eqns. (1.3)–(1.4) it is clear that an increase of the host population size N and/or transmission rate constant β_0 (i.e., contract rate) may dra-

[3]These exclude the presence of within-host competition and density-dependent effects in host reproduction and infection (Nowak and May, 1994; May and Nowak, 1995; Bonhoeffer and Nowak, 1994b; Dieckmann, 2002).

matically increase optimal virulence of epidemic infections but not of endemic infections (Frank, 1996).

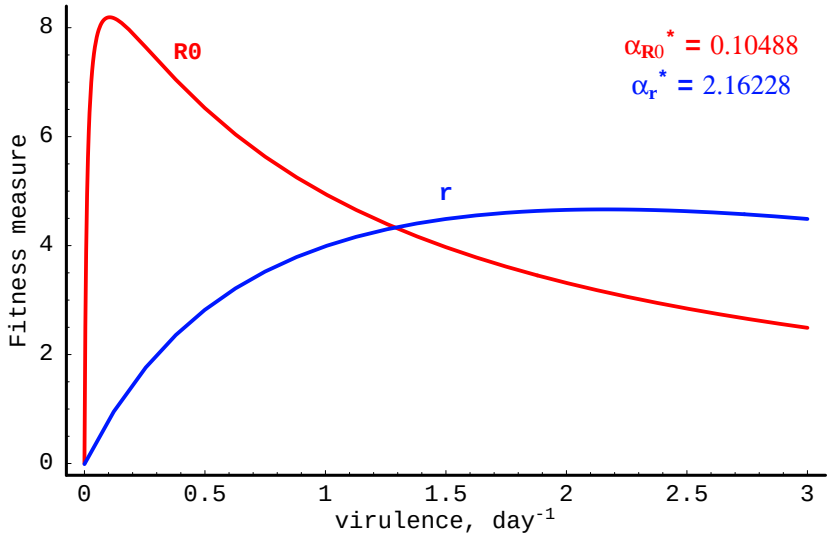

Figure 1.3: The relationship between the basic reproductive number R_0, the net growth rate of the epidemic r and the parasite-induced host mortality rate α. The correlation between the transmission rate and virulence is assumed in the form $\beta = \beta_0\alpha/(\alpha + c)$. Parameters are: $\beta_0 = 0.01$, $d = 0.001$, $c = 1$, $\nu = 0.01$, $N = 10^3$. Optimum levels of virulence for epidemic and endemic diseases are: $\alpha^*_{epidem} \approx 2.16$ and $\alpha^*_{endem} \approx 0.11$. As shown in the Appendix if $\nu = \text{const}$ then for any trade-off $\beta = \beta(\alpha)$, $\alpha^*_{epidem} > \alpha^*_{endem}$.

As far as I know there have been no studies designed to test this prediction of the adaptive theory. There are, however, several observations that are consistent with it. First includes the increase in virulence of parasites in SPEs (Ebert, 1998). In these experiments, parasites are generally transmitted early in the infection thus mimicking the initial phase of an epidemic. On the other hand, selection for later transmission in SPEs that mimics an endemic leads to reduced virulence (Dobson and Owen, 1977; Elena, 2001; Cooper et al., 2002). For a more general discussion on how timing of transmission may affect the optimal level of parasite virulence, see (Day, 2003).

Second, it has been widely argued by Paul Ewald that higher host densities and high rates of parasite transmission are responsible for outbreaks of highly virulent parasites

including influenza, cholera and HIV (Ewald, 1991, 1994). Although these arguments are consistent with the theoretical analysis, experimental data used in support of this prediction may have other interpretations (Frank, 1996). In addition, there are additional reasons to question the generality of these predictions applied to these particular infections as the absence of clear trade-offs between virulence and other traits (Ebert and Bull, 2003).

HIV in particular has drawn much attention in part because of its extreme lethality. For example, for HIV in particular and for sexually transmitted diseases (STDs) in general, it has been suggested that an increased contact rate may select for lower virulence because of the law of diminishing returns (Lipsitch et al., 1995a). STDs should also be less virulent if they reduce sexual activity of the host (Knell, 1999). Why HIV is virulent still remains an open question. Ewald suggested that HIV evolved from a less virulent form because of increased contact rate (Ewald, 1991). Obviously, this need not be the case, because rhesus macaques infected with SIVsm/HIV-2, a natural parasite of sooty mangabeys, progress rapidly to AIDS and die (Staprans et al., 1999; Buckley et al., 2003).

We can however ask a question of whether HIV will evolve to a more/less virulent form (Ganusov, 2003). Levin and Bull (1994) suggested that host immunodeficiency due to HIV infection may result from the within-host, short-sighted evolution of the virus and may have nothing to do with the rate of virus transmission (see Section 1.3.3). Therefore, according to this hypothesis changes in opportunities for transmission should not affect the optimal virulence of HIV.

However, it has been shown that the duration of HIV infection is inversely correlated with the virus density in plasma of infected hosts early in the asymptomatic period of the infection called the set-point (Mellors et al., 1996; Arnaout et al., 1999; Staprans et al., 1999; Goto et al., 2002). Similarly, viral load is positively correlated with the probability of heteterosexual transmission of HIV (Pedraza et al., 1999; Quinn et al., 2000; Gray et al., 2001). Such relationships indicate that there might be a positive correlation between HIV transmissibility and virulence (measured as the inverse of the duration of infection) for viral strains with different set-points. Although both host and virus factors play an important

role in determining the rate of progression to AIDS (Deacon et al., 1995; Magierowska et al., 1999; Staprans et al., 1999; Theodorou et al., 2003; Buckley et al., 2003), their relative contribution to the disease progression is not yet understood (Theodorou et al., 2003). Formally, the correlation between transmissibility and virulence for HIV strains can be established if genetically identical hosts are infected with different strains of the virus. Given the lack of inbred strains of monkeys, this can be done, for example, by observing the disease progression in identical twins infected with different viral strains (Segal and Hill, 2003).

If the correlation between HIV transmissibility and the duration of infection is established, the adaptive theory suggests that HIV virulence will evolve. The exact value of the optimal level of HIV virulence will be then determined by (1) the exact shape of the correlation, (2) whether it is endemic or epidemic, and (3) what exactly is maximized by the virus (Ganusov, 2003).

For example, if the majority of the virus transmission occurs *only* during the acute infection, then we cannot predict HIV evolution since currently there is no relationship between viral load in acute infection and the rate of progression to AIDS (Staprans et al., 1999). Theoretical analysis suggests that during initial stages of the HIV epidemic the majority of the virus is transmitted early during the infection (Levin et al., 1996, 2001) but the relative contribution of acute infection and asymptomatic phase in such early transmission is not known. If, however, all transmission occurs within the first year of the infection given that the acute phase lasts for approximately one month, higher viral loads (and therefore faster progression to AIDS) in the asymptomatic phase may be advantageous to the virus during an epidemic. Selection (if any) may change as the infection reaches the endemic regime. Thus, predictions on whether an infection becomes more or less virulent depends critically on whether it is epidemic or endemic even if the trade-offs for the infection are established.

1.3.2.4 Within-host competition: mutation, co- and super-infection

Some of the predictions of the adaptive theory given in previous sections are based on the assumption that only one parasite strain can occupy a given host. In many instances this needs not be true. For example, *Daphnia magna* can be repeatedly infected with the same microsporidian parasite (Ebert, 1995). Humans infected with malaria often harbor several different strains of the parasite (Read et al., 2002). There have been several theoretical results suggesting that allowing competition between different parasite stains within one host, will select for increased virulence. Parasites evolve higher virulence in this case because of the risk to share the host with a more virulent parasite strain[4]. The increase in the number of parasite strains occupying the same host may result from mutation (Bonhoeffer and Nowak, 1994b) and co- or superinfection (Sasaki and Iwasa, 1991; Frank, 1992; Nowak and May, 1994; May and Nowak, 1995; van Baalen and Sabelis, 1995; Frank, 1996; Mosquera and Adler, 1998; Leung and Forbes, 1998). Importantly, many predictions of the adaptive theory change if the within-host competition between parasite strains in multiply infected hosts is allowed. For example, higher natural host mortality rate would lead to selection of parasites with lower virulence. This is because with shorter infection there is less chance of multiple infections, and in particular superinfections, to occur (Gandon et al., 2001a).

The prediction of the adaptive theory that, in the presence of within-host competition, parasites should evolve to higher virulence, has been tested in several systems. As described in Section 1.3.2.1, it was hypothesized that multiple infections were responsible for increase in virulence of a microsporidian parasite *G. intestinalis* evolved in a population of *Daphnia magna* with low natural host mortality rate (Ebert and Mangin, 1997). Mixed-clone infections of mice with malaria parasite *P. chabaudi* result in higher maximum weight loss of the host (Taylor and Read, 1998; Read et al., 2002) that correlates well with

[4]In some cases, however, cooperation between different parasite strains may increase the efficacy of host exploration (Turner and Chao, 1999); such parasites are expected to evolve lower virulence (Brown et al., 2002).

other measures of virulence in this experimental system (Mackinnon and Read, 1999). In another experimental system, virulence of parasites infecting fig wasps is determined by how many different wasps (generally infected with distinct parasites) pollinate a given fig. If a fig is pollinated by several wasps, parasites infecting such wasps are on average more virulent than parasites infecting wasps that pollinate only unpollinated figs (Herre, 1993). Higher virulence of such parasites is most likely due to an increased level of competition with unrelated parasites within the same fig (Herre, 1993; Frank, 1996). Finally, the assumption that multiple infections are more virulent was necessary to correctly predict the epidemic of the *Cucumber mosaic virus* in the population of tomato plants (Escrui et al., 2003).

On the other hand, some observations of malaria infection of humans or trypanosome infection of bumblebees suggest that single infections may be as virulent as mixed infections (Imhoof and Schmid-Hempel, 1998; Read et al., 2002).

Clearly how multiple infections occur and the type of infection is important in determining whether and how multiple infections will affect the parasite evolution. For example, during an acute infection, the probability of superinfecting an already infected host is very low because of the short duration of infection. Similarly, because of the short duration of infection, mutations are not likely to generate high diversity in the parasite population during the infection unless the mutation rate is extremely high. Therefore, the presence of different parasite strains in the initial inoculum is the most likely mechanism by which multiple infections may occur in acute infections. In contrast, during chronic infections all the three mechanisms (co-, superinfection and mutation) may lead to increased parasite diversity in infected hosts.

Importantly, for some medically important infections (such as *Plasmodium falciparum* infection of humans), the role of multiple infections on the severity of the disease is not well understood (Smith, 2002; Read et al., 2002). Despite this fact, there have been many theoretical models assuming that multiple infections are the main force in driving the parasite evolution (see, for example, Gandon et al. (2001)). Clearly, more experiments with

particular infections are needed to establish how multiple infections affect the within-host dynamics of different parasite strains, severity of the infection and transmission success of parasites.

1.3.2.5 Host heterogeneity

Many simple predictions of the adaptive theory are based on the assumption that parasites evolve in populations of identical hosts. Clearly this is not the case for any natural population where genotypic, phenotypic and age differences between different hosts exist. Little work has been done to understand how parasites evolve in such heterogeneous host populations.

Omitting many details of how heterogeneity can be generated and maintained (for a review see (Ebert and Hamilton, 1996; Ebert, 1999; Galvani, 2003)), it is generally believed that higher levels of host heterogeneity would select for less virulent parasites (Ebert, 1998, 1999). To reach this conclusion it is implicitly assumed that (1) parasites are not able to adapt to different host types simultaneously[5], and (2) adaptation to one host type such as to increase replication/virulence is traded-off with replication in other host types. Some experimental observations are consistent with the assumptions and the prediction (Ebert and Hamilton, 1996; Ebert, 1998).

For example, spread of infections in host populations with low genetic diversity (such as some human populations or agrocultures) often results in high host moralities (Black, 1992; Ebert, 1999). Similarly, many parasites when serially passaged in new genetically identical hosts (or in hosts with low genetic diversity), evolve to increase their virulence (Ebert, 1998). In accord with this increase, virulence generally decreases when it is measured in the original host (Ebert, 1998).

Importantly, many of these and other observations can be explained without assuming that host heterogeneity selects for parasites with low virulence. For example, high host

[5]By host types I mean different host strains for single-host parasites or different host strains/species for multi-host parasites.

mortalities during an epidemic are expected if there is a correlation between the rate of parasite transmission and virulence (Section 1.3.2.3). An increase in virulence of serially passaged parasites may be simply due to strong selection for more rapid growth and not due to low genetic diversity of hosts (Ebert, 1998). The discrimination between the last two explanations can be done in SPEs if hosts of different genetic backgrounds are being used. If increase in virulence during SPEs is due to low host diversity, changing hosts at random or at each passage should prevent parasites from adapting to one host type and virulence from escalating. If the increase in virulence is due to relaxing the requirement for transmission, virulence should increase despite the hosts being changed.

In one experiment, the latter prediction has been confirmed. Turner and Elena (2000) passaged vesicular stomatitis virus, originally growing in BHK cells, in two novel host cell types (HeLa and MDCK cells). There was an increase in the instantaneous growth rate of the virus when measured in the new cell type and its simultaneous reduction when measured in the original cell type or another cell type. However, when cell types used for passage where changed (at random or after each passage), the evolved strain increased its growth rate in *both* novel cell types but had a reduced growth rate in the original cell type. In another experiment, a strain of *P. chabaudi* that had been passaged in C57Bl/6J mice was more virulent in two unrelated mouse strains, CBA/Ca and DBA/2, than the unpassaged parasite strain (Mackinnon et al., 2002).

Given these contradictory results, there is still no good understanding of how host heterogeneity affects the optimal level of parasite virulence. As far as I know only one theoretical study formally has addressed this question. Ganusov et al. (2002) assumed that parasites causing acute infections in vertebrates evolve in the population of hosts that stochastically differ in their susceptibility to infection or their quality of the immune response. They found that in the absence of heterogeneity parasites evolve to an intermediate growth rate but kill no host. The latter is due to the fact that there is a high loss in total transmission when the parasite kills the host. When the level of host heterogeneity increases, the optimal level of parasite virulence measured as the case mortality

increases as well. This is because in order to obtain the maximum total transmission, the parasite has to compromise between killing "susceptible" hosts and obtaining high transmission from "resistant" hosts (Ganusov et al., 2002). Thus, the analysis suggests that higher levels of stochastic heterogeneity should select for higher optimal level of parasite virulence.

In a recent study it has been proposed that virulence of *Neisseria meningitidis*, infecting an immunologically diverse host population, may be the result of selection for parasites with high mutation rates (Ancel Meyers et al., 2003). A high mutation rate increases the changes of the parasite to infect heterogeneous hosts but simultaneously increases the probability of killing an infected host by evolving highly virulent strains within the host. If this explanation of *N. meningitidis* virulence is correct, it is expected that the parasite, infecting a homogeneous host population, should evolve low mutation rate and consequently low (theoretically zero) virulence.

In contrast, Regoes et al. (2000) have found that when two hosts types are present in the population, parasites evolve lower virulence than when only one host type is present. This is because the authors assumed an explicit trade-of between parasite virulence in two host types. While parasites infecting only one host type may evolve infinite virulence, this explicit trade-off does not allow virulence to escalate when two host types are present (Regoes et al., 2000). This study, however, did not investigate how the *degree* of host heterogeneity affects the optimal level of parasite virulence.

At this point we need more theoretical and experimental studies to answer the question of how host heterogeneity affects the parasite evolution. The term *heterogeneity* may also be interpreted differently whether one considers several strains of one host species or different host species. This becomes particularly important since many parasites infect more than one host species (Woolhouse et al., 2001). We expect however that the answer may be different for different parasite-host associations.

1.3.2.6 Route of transmission

Different parasites have different mechanisms of spreading from infected to susceptible hosts including horizontal (direct, vector-borne, and fecal-oral) and vertical transmission (Anderson and May, 1991; Mims et al., 2001). There has been a great debate pioneered by Paul Ewald on whether the route of parasite transmission may be the most important factor in determining virulence of parasites (Ewald, 1983, 1988, 1991, 1994). The main theme of all these predictions is that increasing opportunities for transmission should select for parasites with high virulence and similarly, reducing opportunities for transmission should select for parasites with lower virulence.

For example, according to this hypothesis, since host mobility is not required and may be deleterious for the transmission of vector-borne parasites, such parasites should on average be more virulent than parasites that are transmitted directly and that require host mobility for transmission (Ewald, 1983). Similar arguments are applied to waterborne infections causing diarrhoea because they can spread from immobilized hosts (Ewald, 1991). Although comparative data on different parasites support this idea, other data, for example, on transmission rates of malaria parasites of lizards that differ in their virulence, do not (Schall, 2002). In addition, such comparative analysis across different parasite species without consideration of other details of infection has other shortcomings (Ebert and Bull, 2003). Theoretical analysis also suggests that whether vector-borne parasites are more virulent than directly transmitted parasites depends critically on the morbidity costs and the time schedule of the parasite transmission and needs not be true in general (Day, 2001, 2002a). On the other hand, assuming a positive correlation between parasite transmissibility and virulence for directly transmitted and waterborne parasites, Ewald and De Leo (2002) have found that parasites that can be transmitted directly through contact and indirectly through environment (such as waterborne parasites) evolve higher virulence than parasites transmitted exclusively directly. In their model, the basic reproductive number of the parasite is composed of two R_0 that are related to each transmission mode and are dependent on parasite virulence α:

$$R_0(\alpha) = R_0^{dir}(\alpha) + R_0^{indir}(\alpha) = \frac{\beta(\alpha)N}{\alpha + d + \nu} + \frac{\beta_w(\alpha)\rho/mN}{\alpha + d + \nu}, \tag{1.5}$$

where β_w is the infection rate of susceptible hosts by parasites in the environment, ρ is the shedding rate of parasites into the environment by infected hosts, and m is the decay rate of the parasite in the environment (i.e., $1/m$ is the parasite longevity in the environment), and other parameters are the same as in eqn. (1.1).

The maximum of the total R_0 can be achieved at virulence levels that are higher or lower than the optimal virulence of parasites transmitted only directly (i.e., when $R_0^{indir} = 0$). The optimal level of virulence depends critically on the relative contribution of two transmission routes and *the trade-offs* imposed for two routes. The authors assumed a bell-shaped trade-off for the direct transmission ($\beta = \beta_1\alpha/(c_1 + \alpha^2)$) and linear or saturating trade-off for the indirect transmission ($\beta_w = \beta_2\alpha$ or $\beta_w = \beta_2\alpha/(c_w + \alpha)$). The verbal argument for choosing these functions is that in directly transmitted infections, higher virulence level while causing initial increase in the transmission rate, will eventually reduce the transmission rate due to host immobilization. Since indirect (waterborne) transmission is not affected by the immobilization, there is no decrease in transmission rate with increasing virulence (Ewald and De Leo, 2002). Unfortunately, to my knowledge there is no experimental data that demonstrate such functional forms of the trade-offs for different routes of transmission for cholera. Experimental tests would have to involve measurement of costs of morbidity for direct transmission and virulence-transmission trade-offs for both routes of transmission.

Nevertheless, at these "appropriately" chosen functions the authors indeed found that parasites that are transmitted both directly and indirectly evolve higher virulence than parasites transmitted exclusively directly. However, using similar trade-offs but changing constants describing the trade-off for indirectly transmitted parasites, I find that optimal virulence may be higher or lower than that for exclusively directly transmitted parasites, depending on the parameter values (Figure 1.4). This example demonstrates the major weakness of the "epidemiological" approach for understanding the evolution of parasites: in

many cases not only the particular biological details of the modeled system are important, but the conclusions can be affected by the exact shape of trade-offs used in the analysis.

Other studies nevertheless suggest that if parasites evolve in a spatially structured host population then an increase in opportunities for infection of hosts distant to the infected host will lead to selection of parasites with higher virulence (Boots and Sasaki, 1999; van Baalen, 2002). This is because when only local transmission is allowed, parasites cannot afford to be too virulent because they could deplete all local hosts before being transmitted to the next host patch. When global transmission is allowed, this requirement is relaxed. As yet, no experimental tests have been done to test these predictions. Theoretically, such tests would involve evolution of parasites, for example, by passaging, in environments with different spatial structures such as test-tubes (global transmission) and petri-dishes (local transmission).

There has been a similar discussion on the relationship between the longevity of parasites in the environment and their optimal virulence level. Ewald has suggested that high longevity of parasites in the environment should select for high virulence, because longer survival in the environment relaxes the parasite need for host and for transmission (Ewald, 1994). Several theoretical studies have attempted to address this question but reached different conclusions. Bonhoeffer et al. (1996) have found that parasite longevity does not affect the optimal level of parasite virulence for endemic infections transmitted exclusively indirectly. Since there is no trade-off between the parasite longevity in the environment and its virulence, parasite longevity affects only the R_0 of the infection but not optimal virulence (Bonhoeffer et al., 1996). Gandon (1998) has found that if multiple infections are allowed, higher parasite longevity will select for higher virulence because of the increased within-host competition between unrelated parasite strains. Finally, Day (2002) has found that in cases when there are both direct host-to-host parasite transmission and indirect infection of hosts by parasites in the environment, better parasite survival correlates with higher optimal virulence. The last prediction, however, similar to the case with direct and indirect transmission routes, depends heavily on the appropriately chosen trade-offs and

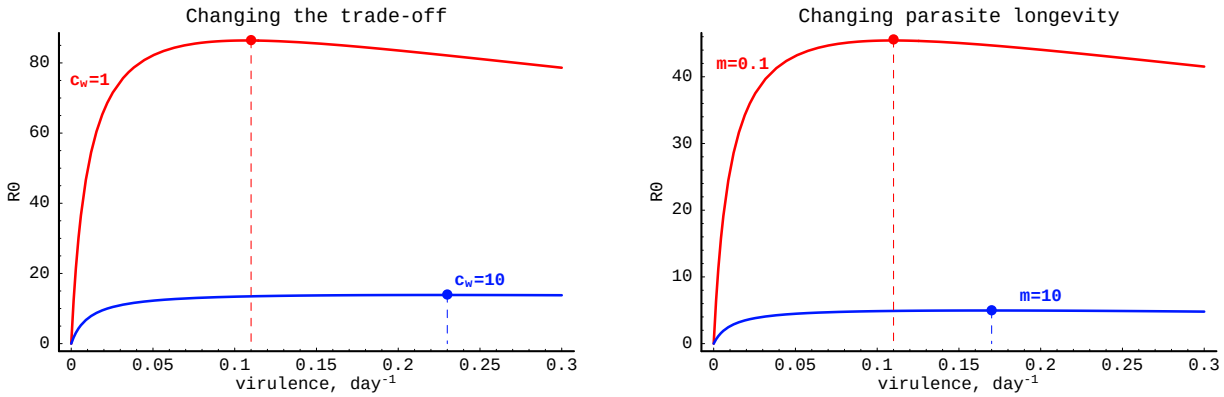

Figure 1.4: The basic reproductive number of an infection transmitted directly and indirectly as the function of virulence α. The correlation between the transmission rate and virulence is assumed to be of the form $\beta = \beta_0\alpha/(\alpha^2 + c)$ and $\beta_w = \beta_0\alpha/(\alpha + c_w)$, for direct and indirect transmission, respectively. The optimum level of virulence for parasites transmitted only directly $\alpha^*_{direct} \approx 0.18$. The basic reproductive number is calculated in accord with eqn. (1.5). Left panel shows changes in the optimal level of virulence if the trade-off for indirect transmission is changed: $c_w = 1$ ($\alpha^* \approx 0.11$) and $c_w = 10$ ($\alpha^* \approx 0.23$). Note that depending on the value, parasites transmitted directly and indirectly may evolve higher or lower virulence than parasites transmitted only directly. Right panel shows changes in the optimal level of virulence when the parasite longevity in the environment is changed: $m = 0.1$ ($\alpha^* \approx 0.11$) and $m = 10$ ($\alpha^* \approx 0.17$). Note that in this case, long-lived parasites evolve lower virulence (but the opposite is also possible). If the parasite is transmitted only indirectly, parasite longevity does not affect the optimal level of parasite virulence. Other parameters are: $\beta_0 = 0.01$, $d = 0.001$, $c = c_w = 1$, $\nu = 0.01$, $N = 5\,10^2$, $\rho = 1$, and $m = 0.05$.

needs not be generally true (Figure 1.4). In summary, these results suggest that it is very difficult to make any general predictions on whether there is any relationship between the route of transmission and optimal virulence unless specific details of the infection (such as trade-offs) are known.

In contrast with parasites, transmitted horizontally, it has been argued that vertically transmitted parasites should be less virulent because in this case transmission of the parasite is linked to the survival of the host (Ewald, 1987). There are several experimental observations that are consistent with this prediction. Bull and coworkers in a series of elegant experiments have shown that increasing opportunities for horizontal transmission of a bacteriophage lead to selection of more virulent viral stains (Bull et al., 1991; Bull and Molineux, 1992; Messenger et al., 1999). Similar results have been obtained for a plasmid evolving in bacteria (Turner et al., 1998). In another experiment, lymphocytic choriomeningitus virus has evolved in a mouse stock from a relatively virulent horizontally transmitted infection to asymptomatic vertically transmitted disease (Traub, 1939). In an exceptional study by Jeon (1972), an initially virulent bacterial parasite of amoebas became harmless after 5 years of strictly vertical transmission. Importantly, both changes in the parasite and the host were responsible for the reduction in virulence (Jeon, 1972). Finally, a comparative study suggests that vertically transmitted lice are less virulent than horizontally transmitted mites while infecting the same host species, rock doves *Columba livia* (Clayton and Tompkins, 1994).

However, there are examples of apparently only vertically transmitted parasites that yet are very virulent. A microsporidian parasite *Tuzetia sp.* of *Daphnia magna* is a strictly vertically transmitted parasite (at least in the laboratory) but yet it dramatically reduces fitness of infected hosts since infected hosts are outcompeted by uninfected hosts (Mangin et al., 1995). Indeed, if some degree of harm is required for transmission, we expect that even vertically transmitted parasites may be virulent. A fundamental vertical transmission equation suggests that virulent parasites of hosts reproducing sexually, may be maintained in the population while being transmitted exclusively vertically. For that the efficacy of

vertical transmission from both parents must outweight the reduction in host fecundity and survival (Fine, 1975). Another study suggests that while exclusively vertically transmitted parasites should evolve low virulence, even small rates of horizontal transmission may be sufficient for maintenance of highly virulent parasites that are transmitted vertically with high efficiency (Mangin et al., 1995; Lipsitch et al., 1996). Thus the amount of vertical transmission may indicate of how virulent a parasite is but it needs not be the general rule.

1.3.2.7 Other factors

There are other factors that may affect the evolution of parasites and which have not been explicitly discussed here. For example, while simple theory assumes that parasites evolve much faster than their hosts, this may not be entirely correct since many hosts species may increase the rate of their evolution by reproducing sexually (Felsenstein, 1988; Ebert and Hamilton, 1996; Ebert, 1999). Clearly in the presence of parasites, hosts will evolve to become more resistant to the infection and this in turn may affect parasite virulence. One good example comes from the coevolution of the myxoma virus and rabbits in Australia, where as hosts evolved high levels of resistance, the virus evolved higher virulence (Fenner and Fantini, 1999, Figure 1.1). There have been a number of theoretical frameworks attempting to describe how host evolution may occur and what consequences it will have for the parasite evolution (Bowers, 1999, 2001; Gilchrist and Sasaki, 2002; Boots and Bowers, 2003).

Simple theory also assumes that any infected host may in fact infect any susceptible host in the population. While this might be correct for some situations and infections, in general, spatial distribution of hosts may be a critical factor affecting the spread of the infection and consequently the evolution of parasites. Importantly, may predictions of the simple theory cannot be easily extended when there is a spatial heterogeneity in host prevalence. For example, the concept of the basic reproductive number does not work in this case (Haraguchi and Sasaki, 2000; Rauch et al., 2003). Furthermore, parasites may

evolve to high or low virulence depending on particular properties of transmission and host connectivity (Boots and Sasaki, 1999; van Baalen, 2002; Read and Keeling, 2003).

Problems with the adaptive theory of parasite evolution

The predictions of the adaptive theory can be only applied to infections for which the trade-offs between parasite characteristics are known to exist. There is no *a priory* knowledge that such trade-offs exist for *any* parasite-host association. Furthermore, changes in parasite virulence need not be always adaptive to the parasite (Bull, 1994; Levin and Bull, 1994), the fact ignored in many interpretations of experimental results.

In previous sections I have reviewed factors that may influence the evolution of parasites even if we assume that there are trade-offs between parasite characteristics. Importantly, the presence of the trade-offs does not determine the exact level of parasite virulence that depends on the precise functions describing the trade-offs (see, for example, Figure 1.4). In particular, if only the correlation between transmissibility and virulence is known but the trade-off between the host recovery rate and virulence is not, then predictions on how parasites will evolve may be incorrect (Day, 2002b). This can be exemplified by the prediction of the optimal level of the myxoma virus during its evolution in Australia. Based only on the trade-off between the host recovery rate and virus-induced host mortality rate, Anderson and May calculated the optimal level of virus virulence $\alpha^*_{theory} \approx 0.013\ day^{-1}$ that was 3 times lower than the observed value $\alpha^*_{observ} \approx 0.041\ day^{-1}$ (Fenner and Ratcliffe, 1965; Anderson and May, 1982). By adding a trade-off between the rate of transmission and virulence for fleas or mosquitoes, the optimal virulence level became closer to the observed, $\alpha^* \approx 0.040\ day^{-1}$, as shown in Figure 1.2 (Massad, 1987; Dwyer et al., 1990). Given this uncertainty, it seems that it is very difficult to make any strong predictions on the parasite evolution unless details of the specific host-parasite association are taken into account.

A similar result has been obtained by Ganusov and Antia (2003). The authors found that changes in the rate of parasite transmission or the mechanism of the parasite-induced

pathogenesis dramatically alter the optimal level of parasite virulence. Since their approach is based on the assumption that there are trade-offs between parasite transmissibility, host recovery rate and virulence, even though they are given implicitly by the dynamics of the parasite and the immune response (Ganusov et al., 2002), this result emphasizes the role of particular details in predicting parasite evolution.

Another limitation of many mathematical models based on the adaptive theory is that the definition of virulence used may not be widely applied to natural infections (Ebert and Bull, 2003). Virulence is often thought as the property of the parasite but host may play a major role in pathogenesis as well. For example, in infections with noncytopathic viruses pathology may arise because of the immune response destroying virus-infected cells (Wodarz and Krakauer, 2000). For some infections, it is not known whether variation in the disease severity is due to infection with different parasite strains or because of the host variability. The adaptive theory, by focusing only on virulence, ignores the evolution of other parasitic traits such as production of toxins or host castration. Finally, the adaptive theory still does not explain why some parasites are quite avirulent. Can it be that such parasites have evolved to cause little harm in the presence of trade-offs between β, ν and α?

In summary, the adaptive theory of parasite evolution has proven to be a valuable tool in analyzing the evolution of parasites. Yet many limitation of the theory are clear and virulence of many infections is not explained in terms of this theory. Since the field of the evolution of infectious diseases is growing rapidly, future experimental tests will, hopefully, attempt to verify the assumptions behind the adaptive theory as well as test its theoretical predictions for specific infections.

1.3.3 Non-adaptive hypotheses for the maintenance of parasite virulence

While the hypothesis that virulence can be adaptive is very attractive, it is clear that in many cases parasite virulence is not related to parasite's fitness (transmission) and therefore should be considered as *non-adaptive*. In some cases, this is because such parasites infect hosts that are not normally transmit the parasite to other hosts (Mims et al., 2001). Such accidental infections or "spill-overs" may sometimes be very lethal to the host although many harmless infections most likely occur unnoticed (Ebert, 1998). Infections of this type include soil bacteria *Clostridium tetani* causing tetanus, and *Clostridium botulinum* causing botulism. Both parasites cause disease in humans by accident, and toxin production by these bacteria most likely has evolved due to other reasons than to kill humans (Lipsitch and Moxon, 1997). Similarly, hantaviruses, Nipah virus, and rabies may cause serious diseases but yet for neither of the infections there is detectable human to human transmission of the parasite (Chua et al., 2000; Kruger et al., 2001; Mims et al., 2001; Lednicky, 2003). It is possible, however, that such spill-overs may with time evolve to begin spreading from human to human without the requirement for the original hosts. In that case, virulence of such an infection may evolve but how it will evolve would depend on many biological details of the within-host dynamics and epidemiological spread of the parasite.

However, there are some infections, such as *Neisseria meningitidis* and poliovirus, that generally infect many hosts but cause severe disease only in rare occasions (Weiss, 2002). Levin and Bull (1994) have suggested that virulence of such infections may be a result of the short-sighted, within-host parasite evolution. Since natural selection on parasite strains acts not only between infected hosts, but also within the infected host, there might be cases when within-host competition results in emergence of a parasite strain that outcompetes all other strains but leads to the host's death. For example, poliovirus is transmitted through the oral-fecal route and generally does not induce disease. Sometimes, however, it can be

passed into the blood and then into the central nervous system (CNS) where it can cause poliomyelitis. Since there is no apparent transmission of the virus from CNS, the authors argued that virulence of poliovirus results from the its within-host evolution (Levin and Bull, 1994). Similar arguments are applied to *N. meningitidis* causing meningitis and HIV causing AIDS.

Since the exact mechanisms by which these parasites cause the disease are not yet understood, such interpretation of parasite virulence has been questioned (Frank, 1996; Ebert, 1999). For example, Ebert (1999) argues that within-host evolution of highly virulent parasite strains may be the direct cost of having high mutation rate required for evasion of the immune response. Indeed, HIV persists for long periods of time in a given host and during that time it is faced with a constant pressure from the immune system. High mutation rate might be one way of avoiding the recognition by the immune response (Ploegh, 1998). On the other hand, a high mutation rate may have a cost of generating mutants that are able to end the infection by killing the host (Nowak et al., 1991). Similar arguments could be applied to *N. meningitidis* causing a long infection and capable of evolving at a high rate due to phase-shifting (Taha et al., 2002). This alternative explanation generates several specific predictions that could be tested experimentally. An increase in the mutation rate of such parasites should lead, on the one hand, to an increased probability of disease occurrence, and on the other hand, to an increased total transmission from hosts that have not developed the disease. Similarly, decrease of the mutation rate should reduce the total transmission of parasites.

Along the same lines, an alternative explanation for the *N. meningitidis* virulence has been suggested in a recent study by Ancel Mayers et al. (2003). The authors presented a mathematical model where high phase-shifting of the bacteria leads simultaneously to a more rapid epidemiological spread of the infection and a higher probability of causing the disease. In this model, the high mutation rate is advantageous for the parasite because it allows the parasite to adapt faster to a heterogeneous host population to establish the initial asymptomatic infection (Ancel Meyers et al., 2003). In summary, regardless

of forces driving evolution of such parasites, within-host evolution may be an important factor affecting virulence of parasites which, when looked from a between-host viewpoint, may appear to be non-adaptive.

1.4 Conclusion

In this paper I have reviewed three hypotheses suggested to explain the variation in virulence level between different parasites. In the past 10 years using mathematical models based mainly on the adaptive theory of parasite evolution, many specific predictions of how parasites should evolve in different conditions have been generated. Some of these predictions have been tested and more are hopefully underway. Although currently this research is addressing mostly an academic question, I am optimistic that in the future as more experimental data become available, it may be possible to generate public health recommendations to help "manage" parasite virulence.

1.5 Appendix

Optimal virulence level of epidemic and endemic infections

Using a model for the epidemiological spread of directly-transmitted diseases where only single infections may occur, during an endemic parasites evolve to maximize their basic reproductive number, R_0 (Bremermann and Thieme, 1989). At the same assumptions, during an epidemic parasites should evolve to maximize the net rate of the growth in the number of infected hosts, r. Using the definitions for R_0 and r given in eqns. (1.1)–(1.2) we obtain the following relationship between two fitness measures:

$$R_0(\alpha) = \frac{r(\alpha)}{\alpha + d + \nu(\alpha)} + 1, \tag{1.6}$$

where I assumed that both R_0 and r depend on virulence measured by parasite-induced host mortality rate α.

I further assume that the trade-offs between transmissibility, recovery rate and virulence are such that the both fitness measures are maximized at intermediate levels of virulence defined in equations:

$$\left.\frac{dR_0(\alpha)}{d\alpha}\right|_{\alpha=\alpha^*_{endem}} = 0, \tag{1.7}$$

$$\left.\frac{dr(\alpha)}{d\alpha}\right|_{\alpha=\alpha^*_{epidem}} = 0. \tag{1.8}$$

Using eqns. (1.6) and (1.7), we obtain

$$\left.\frac{dr(\alpha)}{d\alpha}\right|_{\alpha=\alpha^*_{endem}} = r(\alpha)\left.\left(\frac{1+d\nu/d\alpha}{\alpha+d+\nu}\right)\right|_{\alpha=\alpha^*_{endem}}. \tag{1.9}$$

It is then easy to see that if $(1+d\nu/d\alpha)|_{\alpha=\alpha^*_{endem}} > 0$ then $dr/d\alpha|_{\alpha=\alpha^*_{endem}} > 0$. Because $dr/d\alpha|_{\alpha=\alpha^*_{epidem}} = 0$, and $d^2r/d\alpha^2|_{\alpha=\alpha^*_{epidem}} < 0$ (maximum), $\alpha^*_{epidem} > \alpha^*_{endem}$. Thus if the host recovery rate changes slowly with virulence (i.e., $|d\nu/d\alpha| \ll 1$), the optimal virulence level of epidemic infections is always greater than that of endemic infections for any trade-off $\beta = \beta(\alpha)$.

1.6 Publication status

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Chapter 2

Within host population dynamics and the evolution of microparasites in a heterogeneous host population

Abstract

Why do parasites harm their hosts? The general understanding is that if the transmission rate and virulence of a parasite are linked, then the parasite must harm its host in order to maximize its transmission. The exact nature of such trade-offs remains largely unclear, but for vertebrate hosts it probably involves interactions between a microparasite and the host immune system. Previous results have suggested that in a homogeneous host population in the absence of super- or coinfection, within host dynamics lead to selection of the parasite with an intermediate growth rate which is just being controlled by the immune system before it kills the host (Antia et al., 1994). In this paper, we examine how this result changes when heterogeneity is introduced to the host population. We incorporate the simplest form of heterogeneity — random heterogeneity in the parameters describing the size of the initial parasite inoculum, the immune response of the host and the lethal density at which the parasite kills the host. We find that the general conclusion of the previous model holds: parasites evolve some intermediate growth rate. However, in contrast

with the generally accepted view, we find that virulence (measured by the case mortality or the rate of parasite-induced host mortality) increases with heterogeneity. Finally, we link the within-host and between-host dynamics of parasites: we show how the parameters for epidemiological spread of the disease can be estimated from the within-host dynamics, and in doing so examine the way in which trade-offs between these epidemiological parameters arise as a consequence of the interaction of the parasite and the immune response of the host.

2.1 Introduction

Microparasites depend critically on their hosts to ensure both their livelihood and transmission, yet many are virulent: they cause harm to their hosts. Why do they do this? In some cases virulence is not selected but is simply coincidental; for example, virulence observed in polio may arise due to "short-sighted" evolution of the virus resulting in the infection of neurons even though this does not increase transmission (Levin and Bull, 1994). In other cases transmission and thus reproductive success of the parasite may be linked to the harm that it causes to its host; for example, the transmission rate of the myxoma virus may be positively correlated with its density within the host and the associated formation of lesions (Fenner and Ratcliffe, 1965). While the empirical evidence in favor of this hypothesis remains limited, linkages or correlations of this sort (frequently referred to as trade-offs) have been proposed in a number of experimental systems (Fenner and Ratcliffe, 1965; Schulman, 1970; Anderson and May, 1982; Bull and Molineux, 1992; Ewald, 1993; Ebert, 1994; Ebert and Mangin, 1997; Ebert and Hamilton, 1996; Frank, 1996; Messenger et al., 1999; Mackinnon and Read, 1999). Unfortunately, the precise shape of such trade-offs have rarely been determined (Anderson and May, 1982; Ebert and Hamilton, 1996), and the underlying causes of trade-offs between transmission and virulence are not well understood for the majority of host-parasite systems.

Broadly speaking, theoretical models of virulence evolution have typically employed several different conceptual approaches (for a comprehensive review see Frank (1996))

two of which we would like to emphasize in particular. The first approach is essentially *epidemiological* (Anderson and May, 1991). In this approach parasites evolve to maximize the net reproductive rate R_0, the average number of new infections arising from a single infected host introduced into a wholly susceptible population (May and Anderson, 1983):

$$R_0 = \frac{\beta N}{\alpha + b + \nu}, \tag{2.1}$$

where β is the transmissibility, α, b and ν are the rate constants for the parasite-induced and natural host mortality and recovery, respectively, and N is the density of susceptible hosts. In the absence of superinfection, selection in the parasite population will act to maximize R_0 (May and Anderson, 1983; Bremermann and Thieme, 1989). If α, β, and ν are not associated, this will lead to selection for highly transmissible ($\beta \to \infty$), avirulent ($\alpha \to 0$) parasites. Virulent parasites can evolve if there are trade-offs between α and β or ν, with evolved virulence levels determined by the shape of the trade-off (Anderson and May, 1982; May and Anderson, 1983). Numerous extensions of the basic SIR (Susceptible-Infectious-Recovered, (Bailey, 1975)) approach have explored the way in which virulence evolution is affected by factors such as mutation (Bonhoeffer and Nowak, 1994b; Bergstrom et al., 1999), co- or superinfection (Levin and Pimentel, 1981; Frank, 1992; Nowak and May, 1994; May and Nowak, 1995; Mosquera and Adler, 1998), the mode of transmission (Lipsitch et al., 1995b,a; Bergstrom et al., 1999), host susceptibility (Antonovics and Thrall, 1994; Frank, 1993; van Baalen, 1998; Gandon and Michalakis, 2000), and host heterogeneity (Regoes et al., 2000).

The second approach focuses on the *within-host dynamics* of the parasite. These models typically track the parasite population within individual hosts and determine how factors which influence this within-host dynamics of the parasite will affect both virulence of the parasite and its transmission. The immunological defenses of vertebrates have evolved to combat parasites and may be expected to be one of the most important factors in determining the within-host dynamics of the parasites. Several models have explicitly considered how the interaction between the parasite and the immune response affects

the within-host dynamics and the consequences for the evolution of virulence of parasites (Antia et al., 1994; Bonhoeffer and Nowak, 1994a; Antia and Lipsitch, 1997).

The epidemiological and within-host approaches each have their advantages and shortcomings. The major advantage of the epidemiological approach lies in its generality: painting nature with a broad brush, these models are typically not restricted to single particular infections. This approach has been used in a number of ways. First, this approach can help determine the nature of the trade-offs between α, β and ν from the epidemiology of spread of specific infections such as the myxoma virus (Anderson and May, 1982). Second, one can assume the existence of various trade-offs between virulence and transmissibility, and then explore the population-level consequences of these trade-offs (Anderson and May, 1982; May and Anderson, 1983). Herein lies a major shortcoming of these models, however: since they assume *a priori* that trade-offs exist, they cannot be used to investigate the source and nature of these trade-offs, nor to derive the existence of these trade-offs from basic biological principles. Third, the epidemiological approach can be used to explore how factors such as intra-host competition between parasite strains alter the evolved level of parasite virulence (Levin and Pimentel, 1981; Bonhoeffer and Nowak, 1994b; Nowak and May, 1994; May and Nowak, 1995; van Baalen and Sabelis, 1995; Frank, 1996; Mosquera and Adler, 1998; Gandon et al., 2001a).

Within-host models typically make much stronger assumptions about the details of infection and host response, and as such are necessarily more narrow in applicability. However, in contrast to many epidemiological models, the trade-offs between transmissibility and virulence naturally emerge from the within-host dynamics of the parasite and immune system (Antia et al., 1994). As we show in this paper, these emergent trade-offs can then, in turn, be used to understand the epidemiological properties of the parasite.

Introducing heterogeneity. The evolution of virulence is, at its core, a co-evolutionary process between parasite and host. As such, we might expect that a proper understanding of this process will require consideration of heterogeneity in both the parasite and host populations. Most previous studies have focused on how heterogeneous parasites will

evolve in a population of homogeneous hosts (see e.g. Nowak and May, 1994). More complex extensions of these models also incorporate host heterogeneity with differences in host susceptibility, host recovery rates, and the ability to transmit the parasite. (These and other forms of host heterogeneity have been observed in a number of host-parasite systems; see e.g. (Fenner and Ratcliffe, 1965; Anderson and May, 1982; May and Anderson, 1983; Ebert and Herre, 1996; Zhong and Dobson, 1996; Woolhouse et al., 1997)). However, these models have largely been aimed at explaining the observed stable polymorphism in host susceptibility and parasite virulence found for many parasite-host systems (Antonovics and Thrall, 1994; Frank, 1993; Gupta and Hill, 1995; Morand et al., 1996). The question of how host heterogeneity affects the evolution of parasite virulence has, until recently, been less thoroughly explored. In a comprehensive review, Ebert and Hamilton (1996) suggested that parasites evolving in heterogeneous populations should evolve to be less virulent than parasites adapting to one host type; a parasite which is specialized to exploit one host type will do poorly at exploiting other hosts. Therefore, if more than one different host population is infected by a particular parasite strain, virulence cannot increase in *all* of the infected populations simultaneously. Regoes et al. (2000) derived a similar conclusion from a theoretical model of parasite evolution in heterogeneous populations, and further explored how — depending on the trade-off between virulence of the parasite in two host types — parasites may evolve to be generalists, infecting hosts of both types equally well, or specialists, infecting one host type to the exclusion of the other.

The addition of heterogeneity to the within-host models of acute infections is particularly important because the simple model predicts that in order to maximize transmission, the growth rate of the parasite will evolve to be sufficiently high that maximum parasite density falls just short of the lethal density (the density of the parasite at which it kills the host) before it is cleared by the immune response (Antia et al., 1994). In this case the evolved parasite does not kill its host. Because the optimum is at this knife edge, the introduction of even a little random or stochastic heterogeneity in the parameters could substantially change the level of parasite virulence. Indeed, Antia and Lipsitch (1997)

show that stochastic heterogeneity in the host population results in a decrease in both the optimal growth rate of the parasite and the total transmission. However, they did not consider the consequences of host heterogeneity on the evolution of parasite virulence.

In this paper we introduce a simple type of heterogeneity — *random* heterogeneity in the parameters describing the host response — and explore how it affects parasite evolution. We expect random heterogeneity to be a virtually ubiquitous aspect of the host-parasite interactions. For example, there may be stochastic variation in the initial parasite inoculum, and hosts may, as a result of being exposed to different antigenic and nutritional environments, have small differences in their specific immune responses following infection (Traub, 1936; Fenner and Ratcliffe, 1965; Schulman, 1970; Zhong and Dobson, 1996; Woolhouse et al., 1997). In this paper, we do not consider the sort of large-scale host heterogeneity which results in specialist/generalist trade-offs such that parasite adaptations favored in one host type necessarily engender fitness costs in the other host type(s). We show that, as expected, the introduction of heterogeneity in the host population prevents parasite adaptation to a particular host type and this results in a decrease in the average transmission of the parasite from an infected host. We find, however, that while an increase in heterogeneity initially leads to a decrease in the optimal growth rate of the parasite, this decline is not monotonic — at very high levels of heterogeneity the optimal growth rate begins to increase. The most interesting and surprising results concern the evolution of virulence in heterogeneous populations. We find that contrary to conventional expectations (Ebert and Herre, 1996; Ebert, 1998; Regoes et al., 2000), in our model changes in virulence depend critically on how virulence is measured; virulence measured by the case mortality increases rather than decreases as heterogeneity is increased. Finally we show how the parameters for epidemiological spread of the disease can be estimated from the within-host dynamics of parasites, and in doing so we examine the trade-offs between the epidemiological parameters α, β and ν that result from the within-host model.

2.2 Results and Discussion

2.2.1 Formulation of the mathematical model

We employ a model — introduced by Antia et al. (1994) — that is designed to describe acute infections in vertebrate hosts with rapidly growing microparasites. In this model, the cycle of infection, transmission, and clearance proceeds as follows: (i) Infection is initiated in a new host by a fixed inoculum, P_0, and the parasite population grows exponentially in the absence of a specific immune response. (ii) The presence of the parasite induces a specific immune response in the host which grows by clonal expansion in a parasite-dependent manner, and kills the parasite at a rate proportional to the product of the parasite and immune cells densities. (iii) There is a lethal or "threshold" density of parasite, D, at which infection kills the host. (iv) The rate of transmission of the parasite from an infected host is proportional to the parasite density within the host, and we assume that the parasite is selected to maximize its total transmission from an infected host during the course of the acute infection. Given these assumptions, the rates of change in the densities of the parasite (P) and specific immune cells (X) will be given by the following equations:

$$\begin{aligned} \frac{dP}{dt} &= rP - hPX, \\ \frac{dX}{dt} &= \frac{sXP}{k+P}, \end{aligned} \tag{2.2}$$

where r and s are the maximum growth rates of the parasite and immune cells, respectively, h is the rate constant for clearance of the parasite by the immune response, and k is the parasite density which stimulates immune cells to grow at half their maximum rate. Since we are primarily concerned with the dynamics of parasites during acute infections, we ignore the contraction and memory phases of the immune response which occur following control and clearance of the parasite. Biologically the relative magnitudes of various parameters are (Antia et al., 1994):

$$P_0 \ll k \ll D, \quad hX_0 \ll r, s, \tag{2.3}$$

where $X_0 = X(0)$ is the precursor frequency (the initial number of immune cells specific to the parasite). The within-host dynamics of the parasites with different growth rates are illustrated in Figure 2.1A. We see that: (i) slowly growing parasites are cleared by the immune response before they reach a high density; (ii) parasites with intermediate growth rates reach higher densities but are cleared by the immune response, provided the lethal density D is not exceeded; and (iii) parasites with the highest growth rates reach the lethal density rapidly and kill the host before being controlled by the immune response.

If the rate of parasite transmission is proportional to the within-host density of the parasite, then a plot of the total transmission as a function of the growth rate r of the parasite shows that maximum total transmission occurs at an intermediate growth rate r^* (Figure 2.1B). The total transmission increases with increasing growth rate, provided that the lethal density is not exceeded (i.e. when $r \leq r^*$). At the point where the lethal density is just exceeded, transmission suddenly drops, as there is no transmission from the dead host (we call this drop in transmission "the cost of killing the host"). A further increase in the growth rate leads to a gradual decline in the total transmission. We can estimate the optimal growth rate r^* (at which the total transmission is maximum), and how the total transmission $l(r)$ changes with the growth rate by the following expressions, derived in the Appendix:

$$\left(\frac{D}{k}\right)^s \approx \left(\frac{r^*}{heX_0}\right)^{r^*}, \tag{2.4}$$

and

$$l(r) = c\int_0^\infty P(t)dt \approx c \cdot \begin{cases} \frac{k}{s}\left(\frac{s}{hX_0}\right)^{r/s}\Gamma(r/s), & \text{if } r \leq r^* \\ D/r, & \text{if } r > r^* \end{cases}. \tag{2.5}$$

where e is the base of the natural logarithm. What consequences does this have for the evolution of virulence? The answer depends on how virulence is defined. We consider

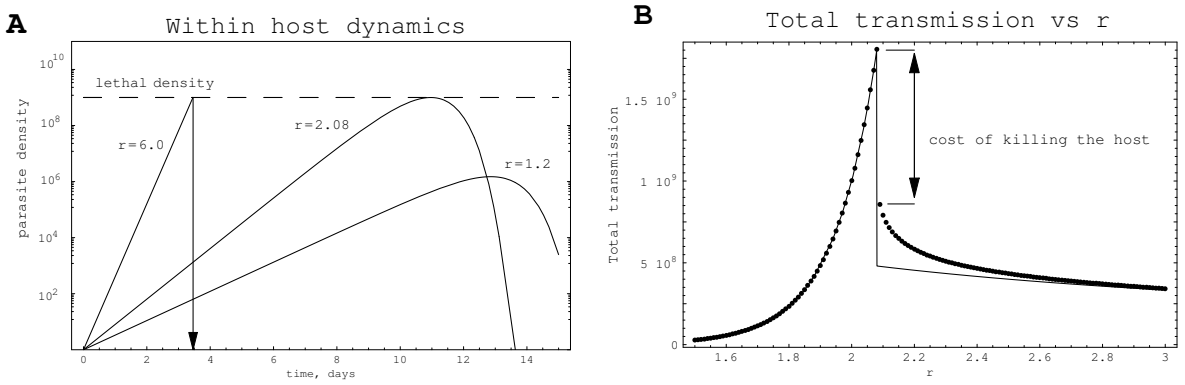

Figure 2.1: The evolution of microparasites in the absence of host heterogeneity. Panel (A) shows the within host dynamics of parasites with different growth rates r. Slowly growing parasites ($r = 1.20$) are cleared by the immune system before they reach a high density, rapidly growing parasites ($r = 6.0$) kill the host before the immune response is activated, and parasites with intermediate growth rates ($r = 2.08$) just approach lethal density D before clearance by the immune system. Panel (B) shows the total transmission of a parasite over the duration of infection as a function of its growth rate r. The maximum total transmission corresponds to the growth rate at which the parasite is just controlled by the immune system before it reaches D. A numerical solution of equations (2.2) ($\cdots$) and analytical approximation given by the equation (2.5) (—) are shown. Parameters: $P_0 = 1$, $X_0 = 1$, $h = 10^{-3}$, $k = 10^3$, $D = 10^9$, $s = 1$.

two commonly used measures for virulence, the LD_{50} and the case mortality[1]. If we use the LD_{50} as the measure of virulence we find that the model predicts (i) the LD_{50} varies inversely with the growth rate r, and (ii) selection for an intermediate growth rate implies selection for intermediate levels of virulence (see Antia et al., 1994). If instead we measure virulence by the case mortality, the model predicts that virulence of a fully evolved parasite (with $r = r^*$) is zero because the maximum parasite density will always fall just short of the lethal density. Because maximum transmission occurs when the maximum parasite density falls infinitesimally short of the lethal density the level of virulence may change dramatically if any heterogeneity is introduced. How it will change is addressed in the next section.

2.2.2 Evolution in a heterogeneous environment

In the simplest model we assumed that, except for the parasite growth rate r, all the parameters describing the parasite-host interaction are fixed. In this section we introduce "random" heterogeneity of the parameters describing the host response, and ask how the optimal growth rate, total transmission, and virulence level of the parasite change with the amount of heterogeneity present in the host population. Heterogeneity in host response to parasite pressure may take a number of (not mutually exclusive) forms. For example, hosts may vary in immune-cell activation rates (different k), frequencies of precursor immune cells (different X_0), immune-cell specificity to the parasite (h), and threshold densities at which parasite load becomes lethal (D). For simplicity, we will begin the analysis by introducing heterogeneity in only one parameter, the threshold density D at which the parasite kills the host. We do so by generating a distribution of threshold densities for a particular host population described by the probability density function $f(D)$ such that $f(D)\ dD$ is the probability that a given host has lethal density in a range $(D, D + dD)$.

[1]The LD_{50} or Lethal Dose 50 is the initial dose of the parasite required to kill 50% of infected hosts in a fixed period of time (Davis et al., 1969). The case mortality is the probability of host death due to the infection.

We consider two simple distributions of the probability density function f: uniform and gamma distributions. We characterize the degree of host heterogeneity by the coefficient of variation $CV = \sqrt{\sigma^2}/\langle D \rangle$, where $\langle D \rangle$ is the mean and σ^2 is the variance of the threshold density. As in the simple model, we assume that different parasite strains differ only in their growth rates r and that r evolves so as to maximize the total transmission over the course of an infection. The average (expected) total transmission of a parasite with growth rate r in this heterogeneous host population is given by the integral of the product of the probability density function $f(D)$ and the instantaneous transmission rate $l(r, D)$ over the course of the infection:

$$L(r) = \int_0^\infty l(r, D) f(D) dD. \tag{2.6}$$

Substituting $l(r, D)$ from the equation (2.5) we find[2]:

$$\begin{aligned} L(r) \;\approx\; & \frac{kc}{s} \left(\frac{s}{hX_0}\right)^{r/s} \Gamma(r/s) \int_{k\left(\frac{r}{heX_0}\right)^{r/s}}^{\infty} f(D) dD + \\ & \frac{c}{r} \int_0^{k\left(\frac{r}{heX_0}\right)^{r/s}} D f(D) dD. \end{aligned} \tag{2.7}$$

In the subsequent sections we explore in detail the consequences of heterogeneity in the parameter D. The discussion of how heterogeneity in other parameters affects the evolution of microparasites is relegated to the end of this section.

2.2.2.1 Transmission and the optimal growth rate

The first two panels of Figure 2.2 provide an overview of the consequences of introducing heterogeneity in the lethal density D. The total transmission changes as a function of growth rate for different levels of uniformly distributed (Panel A) or gamma distributed (Panel B) heterogeneity. Both the total transmission and the growth rate at which transmission is maximized change with increasing heterogeneity. For both uniform and gamma

[2]This approximation has been used to generate the curves in Figures 2.2 & 2.4.

distributions: (i) the total transmission decreases monotonically with increasing heterogeneity and (ii) the optimal growth rate r_{opt} appears to first decrease and then increase as heterogeneity increases. These results are shown in more detail in the second two panels of Figure 2.2. The decrease in the total transmission with increasing heterogeneity is, in retrospect, unsurprising: as heterogeneity increases, the parasite is not able to optimize as well as when there is a single host type. The change in the parasite's optimal growth rate from the value r^* which the parasite has in the absence of heterogeneity to r_{opt} which it has in the presence of heterogeneity is somewhat less intuitive. This change is a consequence of the asymmetry of the total transmission around the optimum r^* observed in Figure 2.1B. At small departures from the growth rate r^* higher transmission is obtained by slightly under-cutting the growth rate r^* ($r_{opt} < r^*$) than by exceeding it. At higher departures from the optimum, higher transmission can obtained by exceeding the growth rate r^* than by under-cutting it. Thus when heterogeneity is low it is on average better to have a slightly lower than the growth rate r^*; when heterogeneity is high it is better to have a higher growth rate.

2.2.2.2 Evolution of virulence

We now examine the effects of host heterogeneity on virulence evolution. We measure virulence in two different ways, as (i) the case mortality, M, and (ii) the LD_{50}. Panel E of Figure 2.2 shows how the case mortality changes with increasing levels of host heterogeneity. For both uniform and gamma distributions of threshold density values, the case mortality increases from zero as heterogeneity increases. When threshold density D is uniformly distributed, there is an initial period when the heterogeneity is low during which there is no increase in the case mortality. When threshold densities are given by a gamma distribution, the case mortality increases almost linearly with increasing heterogeneity. The increase in case mortality is determined by two factors. The optimal growth rate r_{opt} decreases, reducing the maximum density of the parasite within the host. However, heterogeneity in the lethal density D results in some fraction of the host population

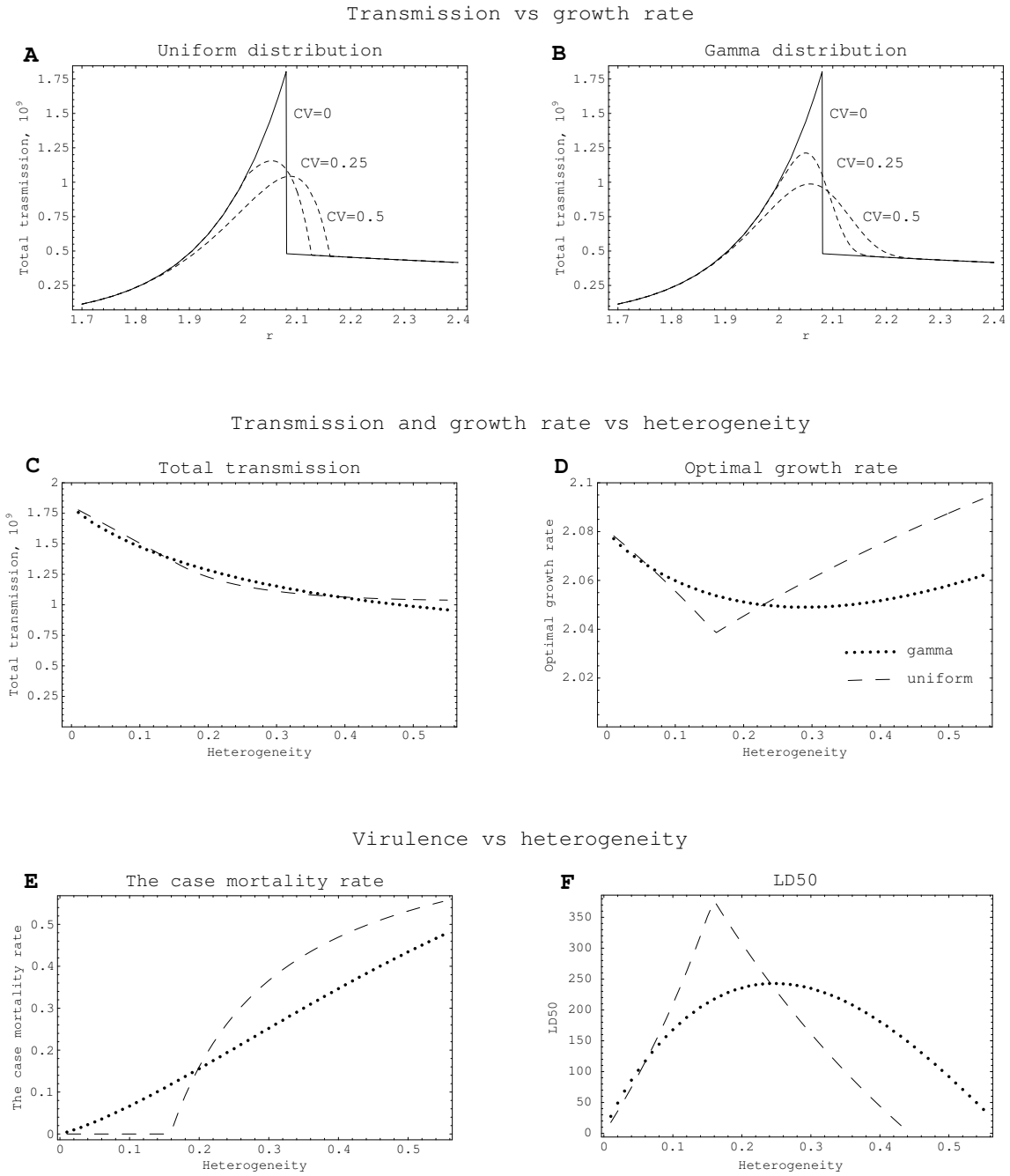

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Figure 2.2: The evolution of microparasites in a heterogeneous host population. We examine the effects of adding heterogeneity in the lethal density D on the evolution of parasites. Panels (A) and (B) show the changes in the total transmission of parasites with different growth rates r when host heterogeneity in the lethal density D is modelled by uniform or gamma distributions, respectively, in the absence (—) and presence (- - -) of heterogeneity (CV is marked). Panels (C) through (F) show the consequences of heterogeneity in the lethal density D for the total transmission (Panel C), the optimal growth rate (Panel D), case mortality (Panel E) and LD_{50} (Panel F) (host heterogeneity is modelled by uniform ($- - -$) or gamma ($\cdots$) distributions). See appendix for details of the calculations. Parameters: same as in Figure 2.1 with $\langle D \rangle = 10^9$.

having a sufficiently low lethal density D that they are killed before the immune response controls the parasite.

In other words, the case mortality increases with increasing heterogeneity as a consequence of the following two changes. First, increasing heterogeneity in D (when the parasite growth rate r is fixed and is below the optimal value r^* given by equation (2.4)) results in an increasing fraction of hosts having a peak parasitemia above the lethal density D; this leads to increasing case mortality. Second introducing heterogeneity changes the optimal growth rate r_{opt} ($r_{opt} = r^*$ when heterogeneity is zero). All else being equal, decreases in r_{opt} lead to a reduction in the peak parasitemia; this leads to decreasing case mortality. As heterogeneity increases from zero to higher values these two changes pull the case mortality in opposite directions, and for non-linear equations we find it hard to intuit the net result. The results of our analysis show that the net effect is an increase in case mortality with increasing heterogeneity.

Panel F of Figure 2.2 shows how the LD_{50} changes with increasing heterogeneity. In the absence of heterogeneity the LD_{50} is just above the initial parasite density, P_0.

As heterogeneity increases, the LD_{50} first increases and then decreases. Changes in the LD_{50} arise as a consequence of the changes in the optimal growth rate of the parasite as heterogeneity increases; the LD_{50} varies inversely with the parasite's growth rate r (see Appendix for details).

Taken together, Panels E and F highlight the critical dependence of our qualitative conclusions regarding virulence evolution on the way in which we choose to measure virulence. The case mortality is the more appropriate measure of virulence as it more closely reflects the actual host mortality caused by the infection and thus the reduction in the host's fitness (i.e. virulence) whereas the LD_{50} is an indirect measure of virulence. In addition, our results show clearly that virulence of a parasite measured by the case mortality does not necessarily decrease as host heterogeneity increases.

2.2.2.3 Heterogeneity in other parameters

We now briefly describe the consequences of introducing heterogeneity in the other parameters describing the interaction of the parasite with the immune response. We do so by looking at how the introduction of heterogeneity in the other parameters changes the total transmission, the optimal growth rate of the parasite and its virulence (Figure 2.3).

As it can be seen in Figure 2.3 the addition of heterogeneity in the parameters h, X_0, and k gives similar results to those arising from variation in D described previously.

The addition of heterogeneity in the size of initial inoculum, P_0, leads to much smaller changes in the total transmission, the optimal growth rate of the parasite and its virulence. While these changes are too small to be seen when plotted with changes in the other parameters they follow the same trend (results not shown). For example, total transmission decreases monotonically with increasing heterogeneity in P_0. In contrast the addition of heterogeneity in the growth rates r leads to larger changes (in total transmission, optimal growth rate and virulence) in comparison with those observed following the addition of heterogeneity to D.

Only in the case of heterogeneity in the maximum growth rate of immune cells, s, we

find that while the behavior is qualitatively similar to that for the other parameters at low levels of heterogeneity, it is qualitatively different when heterogeneity is large. The difference arises because when heterogeneity in s is large there are some hosts with very small s, and in these hosts the immune response develops very slowly, and allows for prolonged transmission of parasites with low rates of growth r. Consequently at very high levels of heterogeneity in s the optimal growth rate r_{opt} declines, the total transmission begins to increase and the case mortality drops. We note that when s is small the infection ceases to be an acute infection of short duration.

2.2.3 Estimating epidemiological parameters

In the Introduction, we contrasted the within-host approach taken in Sections 2.2.1 and 2.2.2 with the epidemiological approach commonly employed to study virulence evolution. In this section, we derive the connection between these two modeling approaches, and demonstrate that the epidemiological parameters of parasite transmission depend explicitly on the within-host dynamics. From the within-host dynamics, we can calculate the epidemiological parameters which define the basic reproductive rate of the parasite (equation (3.1)) — namely the rate of parasite transmission from infected to susceptible hosts, β, and the rates of parasite-induced host mortality, α, and recovery, ν, — and examine the resultant trade-offs between these epidemiological parameters. The epidemiological parameters can be obtained from the within-host dynamics as follows.

First, the basic reproductive number, R_0, is proportional to the average number of parasites transmitted from an infected host over the course of acute infection, i.e. $R_0(r) = uL(r)$, where u is a coefficient of proportionality. Second, the transmission rate of a parasite with the growth rate r from a host with a lethal density D equals the total transmission of the parasite over the course of acute infection, $l(r, D)$, divided by the duration of acute infection, $\Delta(r, D)$:

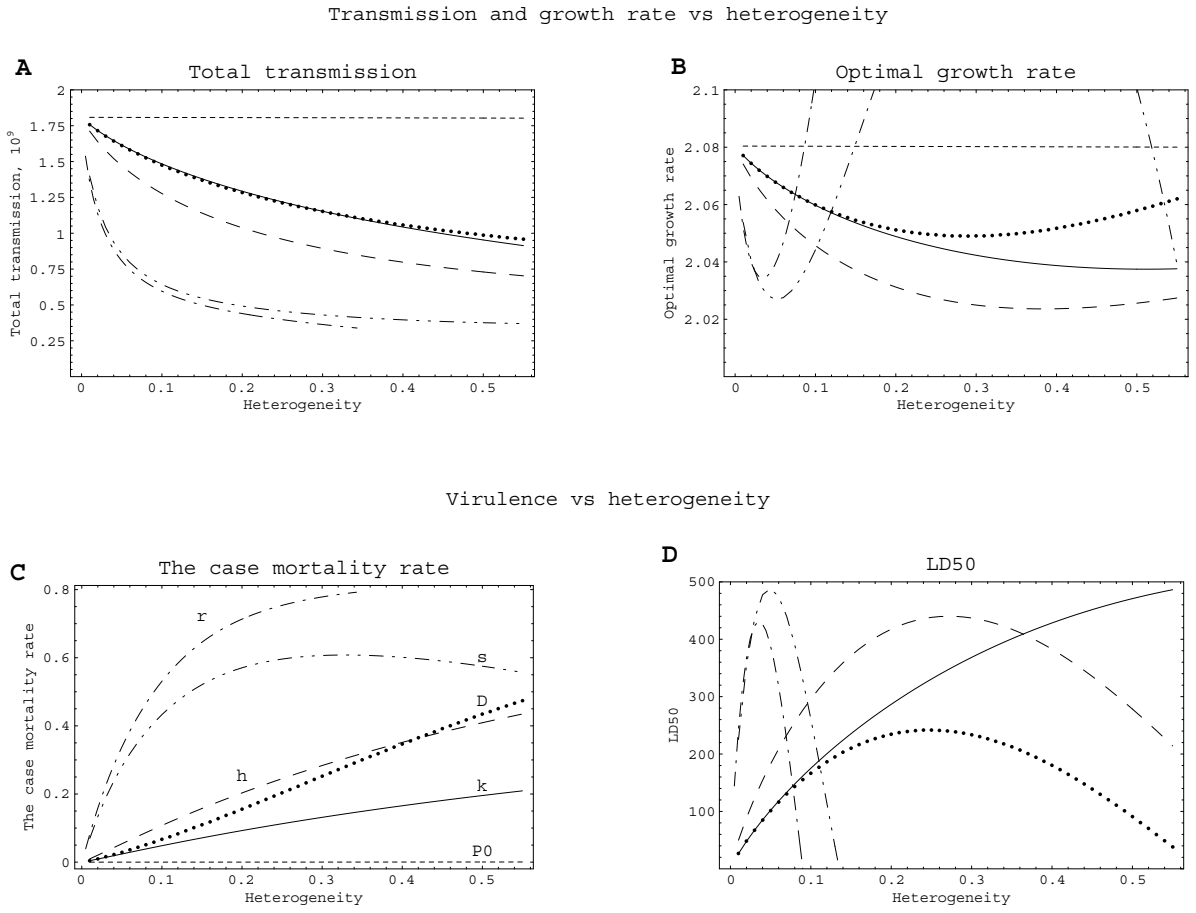

Figure 2.3: Consequences of heterogeneity in other parameters on the evolution of microparasites. We compare the consequences of heterogeneity in the different parameters for the total transmission (Panel A), the optimal growth rate (Panel B), case mortality (Panel C) and LD_{50} (Panel D). Heterogeneity in D $(\cdots)$, k (—), $h(X_0)$ $(---)$, P_0 (- - -), r $(- \cdot -)$, and s $(- \cdots -)$ is modelled by a gamma distribution. Parameters: the same as in Figure 2.1 with $\langle P_0 \rangle = 1$, $\langle X_0 \rangle = 1$, $\langle h \rangle = 10^{-3}$, $\langle k \rangle = 10^3$, $\langle D \rangle = 10^9$, $\langle s \rangle = 1$.

$$\beta(r, D) = u\frac{l(r, D)}{\Delta(r, D)}, \tag{2.8}$$

where $l(r, D)$ is given by the equation (2.5) and $\Delta(r, D)$ is derived in the Appendix. The average transmission rate of the parasite, $\beta(r)$, from the host population with heterogeneity described by the distribution $f(D)$ is:

$$\beta(r) = \int_0^\infty \beta(r, D)f(D)dD = u\int_0^\infty \frac{l(r, D)}{\Delta(r, D)}f(D)dD. \tag{2.9}$$

The mortality rate, α, equals the rate of parasite-induced host death following infection. If hosts die following infection then α is inversely proportional to the average duration of the infection. If hosts survive the infection then α is zero. If $m(r, D)$ equals the case mortality (derived in the Appendix) and $\Delta(r, D)$ equals the duration of infection we get

$$\alpha(r, D) = \frac{m(r, D)}{\Delta(r, D)}. \tag{2.10}$$

Similarly, the recovery rate of hosts with a lethal density D infected with a parasite with growth rate r is

$$\nu(r, D) = \frac{1 - m(r, D)}{\Delta(r, D)}.$$

The average recovery and mortality rates of the host population with heterogeneity described by the distribution $f(D)$ are found similarly to the average transmission rate $\beta(r)$:

$$\alpha(r) = \int_0^\infty \alpha(r, D)f(D)dD = \int_0^{D^*} \frac{f(D)}{\Delta(r, D)}dD, \quad \nu(r) = \int_{D^*}^\infty \frac{f(D)}{\Delta(r, D)}dD, \tag{2.11}$$

where $D^* = k\left(\frac{r}{heX_0}\right)^{r/s}$.

Using the derived expressions for the epidemiological parameters we illustrate (i) how β, α, and ν change as a function of the growth rate of the parasite r when host heterogeneity is held at a fixed level; (ii) what are the trade-offs between these parameters for parasites with different growth rates (Figure 2.4).

Panel A of Figure 2.4 shows the virulence and recovery rates. We see that the mortality rate α increases with the parasite's growth rate r; the rate of increase is greatest when r is close to its optimal value at a given level of host heterogeneity ($r_{opt} \approx 2.06$ for $CV_D = 0.50$). The average rate of recovery ν initially decreases only gradually with increasing r, as the duration of infection gets slightly shorter. Thereafter, as more hosts die, the rate of recovery rapidly declines. Panel B of Figure 2.4 shows that the transmission rate, $\beta(r)$, increases with r until the growth rate at which transmission is maximum, and thereafter drops to $u\langle D\rangle / \ln\langle D\rangle$. We note that since $R_0(r)$ is directly proportional to the total transmission, the change in this measure as a function of growth rate r corresponds to the graph for the total transmission, $L(r)$, as shown in Figure 2.2 Panels A&B.

Finally, we visualize some of the trade-offs between the basic reproductive number R_0, the mortality rate α, and the recovery rate ν. We focus on those trade-offs which have been explored in the epidemiological literature (Fenner and Ratcliffe, 1965; Anderson and May, 1982). In Panel C of Figure 2.4 we show how the basic reproductive number R_0 changes with the level of virulence α. We find that R_0 is maximized at intermediate levels of virulence. In Panel D we show the trade-off between virulence and the recovery rate, and find a nearly-linear relationship between these parameters. The shapes of both these trade-offs are qualitatively consistent with those estimated from experimental data of the myxomatosis infection in rabbits in Australia (Fenner and Ratcliffe, 1965; Anderson and May, 1982).

2.3 Summary

In this paper we have used a simple mathematical model to analyze how host heterogeneity affects the within-host dynamics and evolution of microparasites in vertebrate hosts. We introduce a simple form of heterogeneity, the "random" heterogeneity which arises inevitably in host populations due to factors such as stochastic variation in the initial density of the parasite, the precursor frequency of immune cells specific for the parasite,

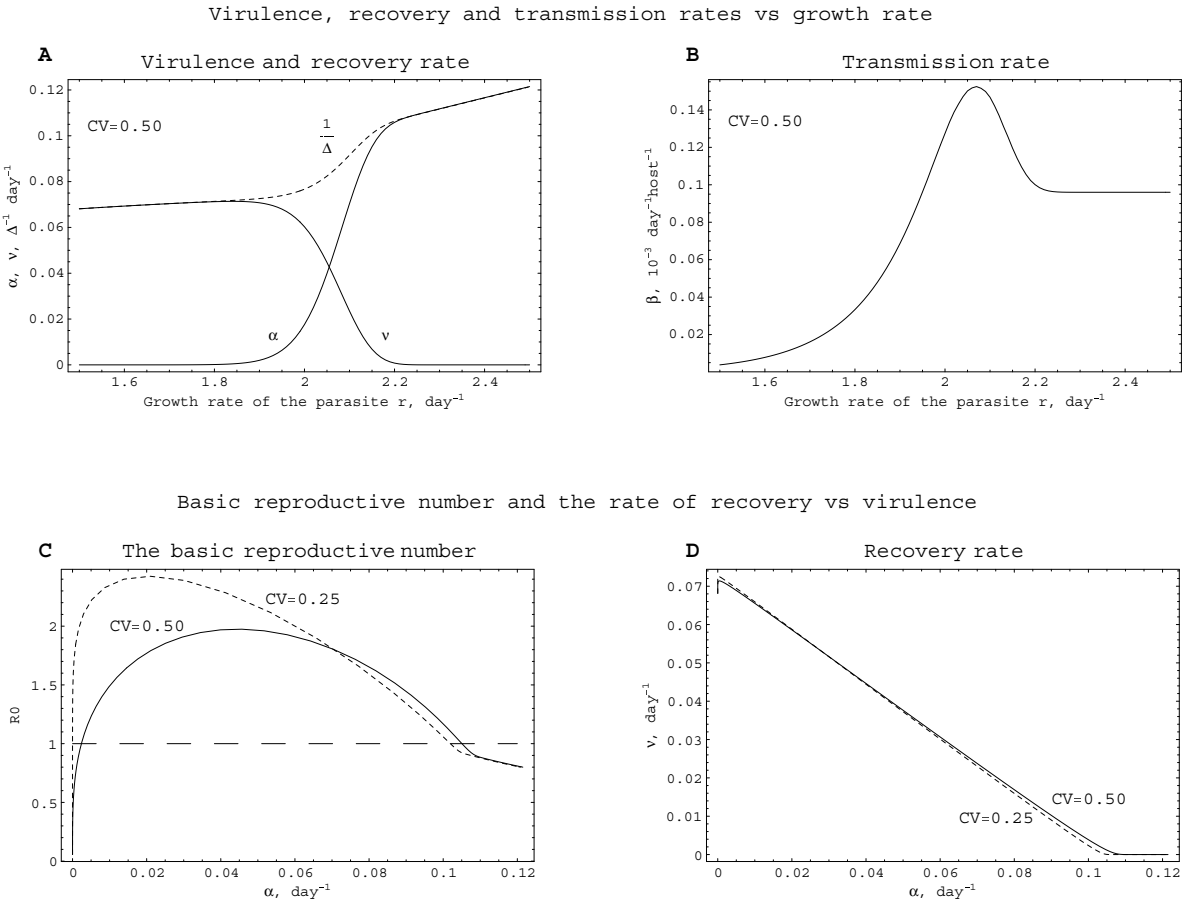

Figure 2.4: Estimating epidemiological parameters from the within-host model. Panels (A)–(B): we estimate the epidemiological parameters for parasites with different growth rates in a heterogeneous host population ($CV = 0.5$). Panel A shows the parasite-induced host mortality rate, α, and the rate of host recovery, ν. Panel B shows the transmission rate, β. Panels (C)–(D) show the epidemiological trade-offs arising as a consequence of the within-host dynamics for two levels of host heterogeneity (with $CV = 0.5$ (—) and $CV = 0.25$ (- - -)). Panel C shows how the basic reproductive number, R_0, changes with virulence, α. Panel D shows the trade-off between the recovery rate, ν, and the parasite-induced host mortality rate, α. Parameters: the same as in Figure 2.2 except $N = 10^3$ and $u = 2 \cdot 10^{-12}$, host heterogeneity in D is modelled by a gamma distribution.

as well as the parameters h, k, and D which determine the within-host dynamics of the parasite. We find that: (i) parasites evolve to an intermediate growth rate, which changes with increasing heterogeneity; (ii) the total transmission of the evolved parasite decreases as heterogeneity is increased; (iii) the observed pattern of virulence evolution is sensitive to the measure of virulence employed (case mortality versus LD_{50}, the former of which provides the preferable measure); (iv) in contrast to the generally accepted view, virulence does not necessarily decrease with increasing heterogeneity, and indeed is likely to increase with increasing heterogeneity; (v) the parameters for epidemiological spread of the disease can be estimated from the within-host dynamics, and in doing so we can examine the trade-offs between these epidemiological parameters that result from the interaction of the parasite and the immune response of the host.

Our finding — that parasites should cause more damage to the host population when hosts are heterogeneous — seems to contradict the widespread belief that the evolution of parasites in heterogeneous populations should select for less virulent parasites (Ebert and Herre, 1996; Ebert, 1998; Regoes et al., 2000). The different predictions of the earlier papers and our model arise from the way heterogeneity is introduced. In the previous papers heterogeneity in the host population *implicitly* imposes a trade-off on the parasite. The consequence of this trade-off is that doing well in one type of hosts impairs the fitness of the parasite when it infects the other type of hosts. In our model we only introduce random or stochastic heterogeneity in the parameters (i.e. we do not impose any specific trade-offs).

In reality we could find both types of heterogeneity. Random heterogeneity (i.e. small differences in parameters governing the host-parasite interactions) is expected to be present in any host population due to phenotypic (or other) differences between individual hosts. When this happens our model predicts that virulence, measured by the case mortality rate, will increase with increasing random heterogeneity. "Trade-off" heterogeneity may be found in other cases. The evidence, suggesting that "trade-off" heterogeneity exists, includes serial passage experiments where the increase in virulence of parasites when they

are serially passaged through one host type is frequently linked with a decrease in the parasites virulence in another host type (Ebert, 1998). Ultimately, our understanding of virulence evolution will benefit from incorporation of both *random* and *trade-off* forms of heterogeneity into evolutionary models, so as to assess the relative importance of these two sources of variation.

2.4 Appendix

The optimal growth rate of the parasite and the total transmission of the parasite from an infected host. By dividing first equation of the system (2.2) by second and integrating we obtain:

$$\log\left(\frac{P+k}{P_0+k}\right) = \frac{r}{s}\log\left(\frac{X}{X_0}\right) - \frac{h}{s}(X - X_0). \tag{2.12}$$

Noting that the maximal transmission occurs when $P(t)$ approaches the lethal density D, we set $P'(t) = 0$ and obtain $X(t) = r/h$. Since the maximal transmission occurs at $r = r^*$, we find the equation (2.4) with the use of the equation (2.12) at the limits $P_0 \ll k$, $D \gg k$ and $hX_0/s \ll 1$. When the total transmission of the parasite from an infected host is proportional to the total density of the parasite we obtain:

$$l(r) = c\int_0^\infty P(t)dt = c\int_{X_0}^{X_{max}} \frac{P(t)+k}{sX}dX = \frac{c(P_0+k)}{hX_0}e^{hX_0/s}\left(\frac{s}{hX_0}\right)^{r/s-1}\int_{hX_0/s}^{hX_{max}/s} y^{r/s-1}e^{-y}dy. \tag{2.13}$$

At the approximations $P_0 \ll k$ and $hX_0/s \ll 1$ the equation (2.13) can be simplified:

$$l(r) = \frac{kc}{s}\left(\frac{s}{hX_0}\right)^{r/s}\int_0^{hX_{max}/s} y^{r/s-1}e^{-y}dy. \tag{2.14}$$

When $r < r^*$ (r^* defined by the equation (2.4)), an infected host clears the infection by a greatly expanding population of immune cells; therefore, at the approximation $hX_{max}/s \gg 1$ we obtain:

$$l(r) \approx \frac{kc}{s}\left(\frac{s}{hX_0}\right)^{r/s}\int_0^\infty y^{r/s-1}e^{-y}dy = \frac{kc}{s}\left(\frac{s}{hX_0}\right)^{r/s}\Gamma(r/s) \tag{2.15}$$

where $\Gamma(x)$ is the Euler gamma function (note that $\Gamma(x) = (x-1)!$ for integral x). When $r \gg r^*$, we can assume that immune cells do not expand; approximating $\exp(-y)$ at $y \in (0, hX_{max}/s \ll 1)$ as 1, we obtain the following expression for the total transmission of the parasite from an infected host at $r \gg r^*$:

$$l(r) = c\frac{D}{r}. \tag{2.16}$$

The duration of acute infection. We consider the duration of acute infection for the cases when $r < r^*$ and $r > r^*$ separately (r^* is given by the equation (2.4)).

If $r > r^*$ the parasite kills the host; approximating that the parasite population grows exponentially until it kills the host, the duration of acute infection $\Delta(r, D)$ will be:

$$\Delta(r, D) \approx \frac{\ln(D/P_0)}{r}. \tag{2.17}$$

If $r < r^*$ then the parasite is cleared by the immune response; the duration of acute infection is found by integrating the equation for dX/dt in equation (2.2):

$$\Delta(r, D) = \int_{X_0}^{X_{max}} \frac{P+k}{sXP}dX, \tag{2.18}$$

where the relationship between the number of immune cells, X, and parasite density, P, at any given moment is given in the equation (2.12). Substituting $P(X)$ from (2.12) the duration of the acute infection when $r < r^*$ equals:

$$\Delta(r, D) = \int_{X_0}^{X_{max}} \frac{dx}{sx}\left(1 - \frac{k}{P_0+k}\left(\frac{X_0}{x}\right)^{r/s} e^{h(x-X_0)/s}\right)^{-1}, \tag{2.19}$$

where the maximum density of immune cells (X_{max}) is approximately found in the equation: $r\ln(X_{max}/X_0) \approx h(X_{max} - X_0)$. Summarizing, the duration of acute infection is:

$$\Delta(r,D)=\begin{cases}\frac{\ln(D/P_0)}{r}, & \text{if } D<k\left(\frac{r}{heX_0}\right)^{r/s},\\ \int_{X_0}^{X_{max}}\frac{dx}{sx}\left(1-\frac{k}{P_0+k}\left(\frac{X_0}{x}\right)^{r/s}e^{h(x-X_0)/s}\right)^{-1}, & \text{otherwise.}\end{cases} \tag{2.20}$$

If $f(D)$ describes the distribution of lethal densities D in the host population then the average duration of acute infection corresponding this distribution will be:

$$\Delta(r)=\int_0^\infty \Delta(r,D)f(D)dD. \tag{2.21}$$

Virulence defined by the case mortality. The case mortality is the probability of host death due to the infection. In a host population with heterogeneity defined by the $f(D)$, the average case mortality $M(r)$ can be calculated as follows. The maximum density reached by a parasite with the growth rate r during acute infection in the model (2.2) is given by the equation (2.4), i.e., $P_{max}\approx k\left(\frac{r}{heX_0}\right)^{r/s}$. Therefore, hosts with threshold densities less than P_{max} ($D<P_{max}$) will die when infected with such a parasite strain while others will survive. Hence, the case mortality $m(r,D)$ will be:

$$m(r,D)=\begin{cases}1, \text{ if } D<k\left(\frac{r}{heX_0}\right)^{r/s},\\ 0, \text{ otherwise.}\end{cases} \tag{2.22}$$

The average case mortality rate M is simply equal to the fraction of the host population with threshold densities less than the maximum density P_{max} reached by the parasite:

$$M(r)=\int_0^\infty m(r,D)f(D)dD=\int_0^{P_{max}} f(D)dD. \tag{2.23}$$

Virulence defined by the lethal density LD_{50}. The LD_{50} is the initial dose of the parasite required to kill 50% of infected hosts in a fixed period of time (Davis et al., 1969). We derive the LD_{50} when this period of time is greater than (or equal to) the duration of acute infection. The results obtained under this condition are similar to those found when the fixed period is shorter (not shown).

We calculate the LD_{50} as follows. The maximal density obtained by a parasite with growth rate r during the course of acute infection is given in the equation (2.12):

$$P_{max} \approx (P_0 + k) \left(\frac{r}{heX_0} \right)^{r/s}. \tag{2.24}$$

The average case mortality is defined in the equation (2.23), with P_{max} from the equation (2.24). Since the initial size of the parasite population is now variable, we define LD_{50} as the density P_0 at which the case mortality M is one half:

$$LD_{50}(r) = P_0 : \quad \int_0^{(P_0+k)\left(\frac{r}{heX_0}\right)^{r/s}} f(D)dD = \frac{1}{2}. \tag{2.25}$$

Solving this integral equation for a given distribution $f(D)$ provides an estimate for LD_{50}. When the variance of the distribution $f(D)$ is small, the solution of equation (2.25) is approximately:

$$LD_{50}(r) \approx \langle D \rangle \left(\frac{heX_0}{r} \right)^{r/s} - k, \tag{2.26}$$

where $\langle D \rangle$ is the average lethal density in the host population.

2.5 Publication status

Ganusov, V.V., Bergstrom, C.T. and Antia, R. (2002) Within host population dynamics and the evolution of microparasites in a heterogeneous host population. *Evolution* 52: 213–23

Chapter 3

Trade-offs and the evolution of virulence of microparasites: do details matter?

Abstract

Models of the within-host dynamics of parasites have been used to consider the evolution of microparasites causing acute infections in vertebrate hosts. In this paper, we use these models to examine how the level of virulence to which a parasite evolves, depends on factors such as the relationship between parasite density and its rate of transmission from infected hosts, and the mechanism of parasite-induced pathogenesis. We show that changes in the terms describing transmissibility and pathogenesis may lead to dramatic differences in the level of virulence to which a parasite evolves. This suggests that no single factor is likely to be responsible for the differences in virulence of different parasites, and that understanding of the evolution of virulence of parasites will require a detailed quantitative understanding of the interaction between the parasite and its host.

3.1 Introduction

Why are parasites[1] virulent? The adaptive model suggests that the level of virulence of a parasite is a consequence of its evolutionary adaptation to maximize its total transmission from infected hosts (Anderson and May, 1982). While we focus on this adaptive model, we note that there are alternatives — the virulence of some parasites may be coincidental (Bull, 1994; Levin and Bull, 1994; Ebert, 1999; Weiss, 2002), or may depend on the competition between different parasite strains within infected hosts (Frank, 1992; Bonhoeffer and Nowak, 1994b; Nowak and May, 1994; May and Nowak, 1995; van Baalen and Sabelis, 1995).

The adaptive model can be understood in terms of the epidemiology of the infection, and in particular in terms of the basic reproductive number, R_0, of the infection caused by the parasite. R_0 is defined as the number of secondary infections produced by one infected host in a wholly susceptible population (Bremermann and Thieme, 1989; Anderson and May, 1991). In the adaptive model the parasite evolves to maximize its total transmission, and this is equivalent to maximizing R_0 (Bremermann and Thieme, 1989; Anderson and May, 1991). The R_0 of a directly transmitted infection can be written as:

$$R_0 = \frac{\beta N}{b + \alpha + \nu}, \tag{3.1}$$

where β is the average transmissibility of the infection, α and b are the rate constants for the parasite-induced and natural host mortality, ν is the rate of recovery from infection, and N is the density of susceptible hosts. The trade-offs between transmissibility β, host recovery rate ν and virulence α determine the values of these parameters at which R_0 is maximum. The existence of some of these trade-offs is intuitive. For example, pathogens which cause infections with higher parasite densities might be expected to have higher transmissibility (β) and higher virulence (α), suggesting a positive correlation between

[1] We use the term parasites for microparasites such as viruses, bacteria, fungi, and protozoa, and focus on acute infections of vertebrate hosts.

transmissibility and virulence. However, experimental data on the functional form for these trade-offs is relatively limited (Fenner et al., 1956; Fenner and Ratcliffe, 1965; Anderson and May, 1982; Schulman, 1967; Mackinnon and Read, 1999).

Models of the within-host dynamics of parasites have been used to understand how these trade-offs arise as a consequence of the interaction between the replicating parasite and the host immune response as well as the transmissibility of the parasite from the infected host (Sasaki and Iwasa, 1991; Antia et al., 1994; Ganusov et al., 2002; Gilchrist and Sasaki, 2002).

In Section 3.2 we briefly review a simple model for the within-host dynamics of microparasites causing acute infections in vertebrate hosts, and illustrate how this model can be used to understand the evolution of virulence of parasites (Ganusov et al., 2002). In the subsequent two sections we consider how the changes in some aspects of the interaction between the parasite and its host in the model affect the evolution of the parasite, and in particular how they affect the optimal level of virulence to which we expect the parasite to evolve. We consider two specific modifications to the initial model. In Section 3.3 we consider the consequences of changing the relationship between the within-host density of the parasite and its rate of transmission from a linear function to a saturating or an exponentially increasing function. In Section 3.4 we consider the consequences of changing the mechanism of pathogenesis by altering the term for parasite-induced mortality. We find that while these changes do not affect the within-host dynamics of parasites, they can dramatically alter the level of virulence at which the total transmission of parasites is maximized.

3.2 Basic Model

We briefly describe a simple model for the within-host dynamics of microparasite infections of vertebrate hosts (for details see Antia et al. 1994, and Ganusov et al. 2002). The model assumes the following:

1. Infection is initiated by a small dose of parasite $P(0)$ which grows exponentially at the rate r in the absence of the specific immune response.

2. The parasite, $P(t)$, kills the host if its density exceeds a lethal density D.

3. The specific immune response, $X(t)$, is generated by clonal expansion from a population of $X(0)$ precursors at the rate $\frac{sP(t)}{k+P(t)}$.

4. The specific immune response clears the parasite at the rate $hX(t)$.

5. Because we consider only acute infections (i.e., the parasite is cleared) we do not consider the subsequent contraction of the immune response.

6. The rate of transmission of the parasite, $\zeta[P(t)]$, is directly proportional to its density within the host, i.e. $\zeta[P(t)] = P(t)$.

With these assumptions the equations describing the within-host dynamics of the parasite and immune response are:

$$\frac{dP(t)}{dt} = P(t)(r - hX(t)), \quad \text{if } P(t) < D, \tag{3.2}$$

$$P(t) = 0, \quad \text{if } P(t) \geq D, \tag{3.3}$$

$$\frac{dX(t)}{dt} = \frac{sX(t)P(t)}{k + P(t)}. \tag{3.4}$$

The total transmission of the parasite over the course of acute infection (of duration Δ), $l(r)$, is

$$l(r) = \int_0^{\Delta} \zeta[P(t)]dt = \int_0^{\Delta} P(t)dt. \tag{3.5}$$

In Figure 3.1A we show the dynamics of the infection for parasites with different growth rates. In Figure 3.1B we show how the total transmission, $l(r)$, depends on the growth rate, r, of the parasite. We see that slowly growing parasites are cleared before they reach high density, and thus achieve relatively little total transmission. Parasites with an

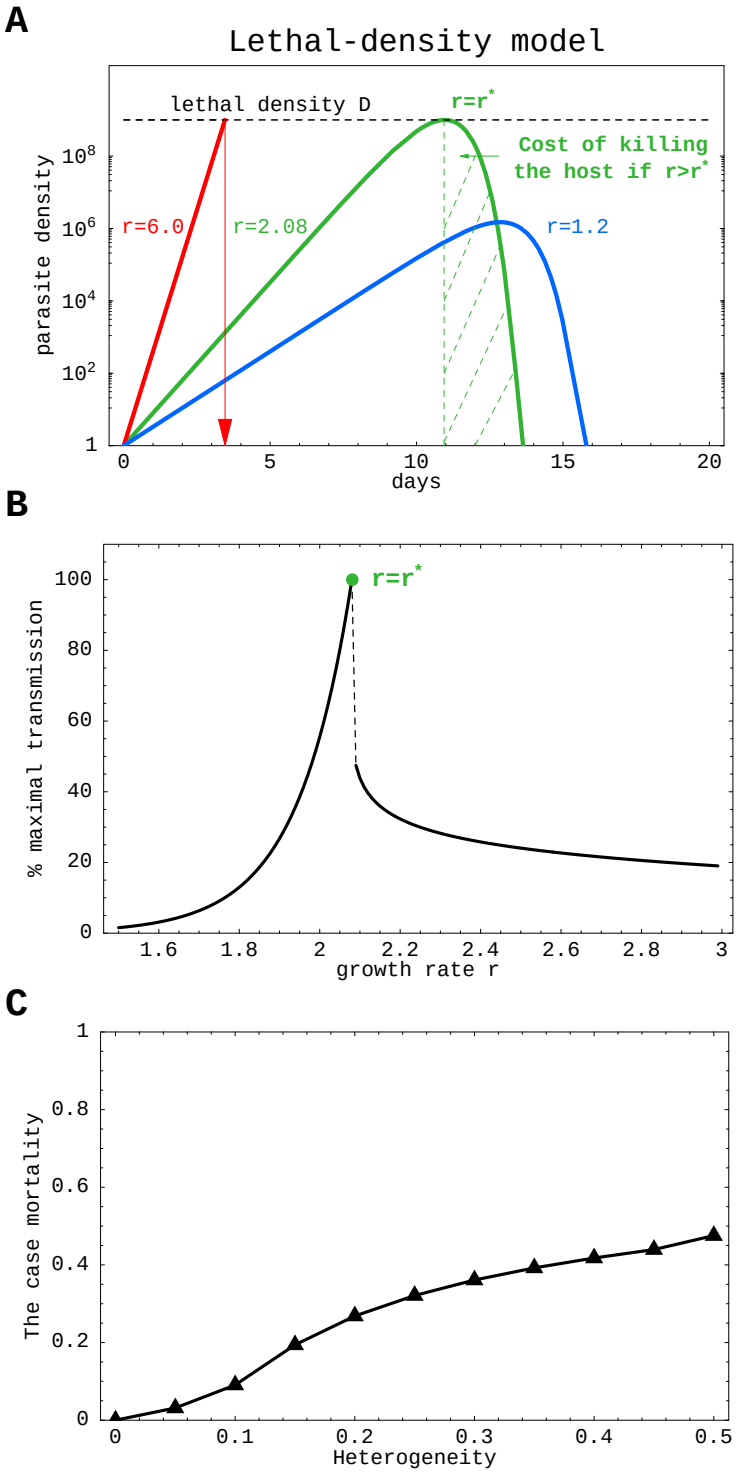

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Figure 3.1: The basic model for the evolution of microparasites. Panel A shows the within-host dynamics of parasites with different growth rates. Panel B shows the total transmission of parasites over the course of acute infection as a function of the growth rate. The dot at $r = r^*$ denotes the optimal parasite. Panel C shows the virulence (the case mortality) of the optimal parasite depends on the degree of heterogeneity. Heterogeneity is described by the coefficient of variation ($CV = \sqrt{variance}/mean$) in the gamma distribution chosen for the lethal density, D. Parameters used for simulations: $P(0) = 1$, $X(0) = 1$, $h = 10^{-3}$, $k = 10^3$, $s = 1$, $D = 10^9$.

intermediate growth rate, r^*, which allows them to reach a maximum density just short of the lethal density before being cleared by the immune response are able to generate the maximum total transmission. Faster growing parasites, which reach the lethal density D, kill the host rapidly and this limits the total transmission of the parasite. These results suggest that evolution will select parasites with an intermediate growth rate.

The model is not able to describe intermediate levels of virulence in a satisfactory manner. This is because all infections are identical and thus either all hosts survive or all hosts die following infection. We note that the virulence measured in terms of the case mortality caused by the evolved parasite is equal to zero. However, an infinitesimal increase in the growth rate from the optimal level will result in the parasite killing all hosts (i.e., having a case mortality equal to 1), and a substantial decline in the total transmission at $r > r^*$ (which we term the cost of killing the host).

This problem can be resolved by introducing stochastic heterogeneity in the host population, and similar results are obtained if we introduce heterogeneity in the any of the parameters (Ganusov et al., 2002). In Figure 3.1C we show how introducing heterogeneity in the lethal density D, results in intermediate levels of virulence (as measured by the case mortality) of the evolved parasite. We find that the level of virulence increases with

increasing levels of host heterogeneity.

3.3 Changing the term for transmission

There is some experimental data suggesting that the rate of transmission of parasites from an infected host, $\zeta(P)$, is positively correlated with the density of parasite within the host, P (Fenner et al., 1956; Schulman, 1967; Taylor and Read, 1997; Pedraza et al., 1999; Quinn et al., 2000). However, the functional form of the term for the rate of transmission has not been quantitatively determined, and furthermore is likely to differ for different infections.

In the earlier model, we chose the simplest possible term, letting $\zeta(P)$ be linearly dependent on P. In this section we consider the consequences of changing $\zeta(P)$ to a function which increases slower than linearly as well as one which increases faster than linearly with increases in P. A slower than linear rate of increase in $\zeta(P)$ with P corresponds to a biological situation where the transmission rate saturates at high parasite densities. A faster than linear increase in $\zeta(P)$ with P corresponds to a biological situation where cooperative effects are needed for parasite transmission.

In the first case, when the transmission rate increases slower than linearly with increases in P, we let $\zeta(P)$ be a simple saturating function

$$\zeta(P) = \frac{P}{1 + P/\theta}, \tag{3.6}$$

where θ is the parasite density at which the transmission rate is half its maximum value. Biologically we expect that $\theta \gg P(0)$.

In the second case, when the rate of transmission increases faster than linearly, we let the transmission rate be proportional to the square of the parasite density:

$$\zeta(P) = P^2. \tag{3.7}$$

We call these two functions which describe the rate of transmission as a function of the within-host parasite density as saturating and squared transmission functions, respectively.

We note that there could be more complex functions for the transmission rate which increase faster than linearly at low densities and saturate at high densities.

In Figure 3.2A we show the dynamics of the infection for parasites with different growth rates. We see that, as might be expected, changing the function describing the transmission rate does not alter the within-host dynamics of infection (plots in Figures 3.1A & 3.2A are identical). In Figure 3.2B we show how the total transmission, $l(r)$, depends on the growth rate, r, of the parasite for the saturating and squared transmission functions. In order to facilitate comparisons we plot the value for the total transmission as a percent of the maximum transmission. We notice that the growth rate r^* of the parasite at which the total transmission is maximum is not altered by these changes in the function describing the transmission rate. When the rate of transmission is a saturating function we find that the peak in the plot of the total transmission as a function of the growth rate becomes broader. When the rate of transmission is a squared function we find that the peak in the plot of total transmission as a function of the growth rate becomes narrower.

In Figure 3.2C we show how virulence of the optimal parasite (measured by the case mortality) changes with the degree of host heterogeneity. We find that for a given level of host heterogeneity, parasites with a saturating transmission function evolve lower virulence and parasites with a squared transmission function evolve higher virulence when compared to parasites with a linear transmission rate. At high levels of heterogeneity, differences in the function describing the transmission rate can result in substantial differences in the level of virulence, as measured by the case mortality. This result occurs because of the shape of the plot of $l(r)$ vs. r. In the case of the "saturating" transmission function, as heterogeneity increases, the parasite obtains more total transmission if it errs on the side of having a slower growth rate compared with r^*. The converse holds in the case of the "squared" transmission function.

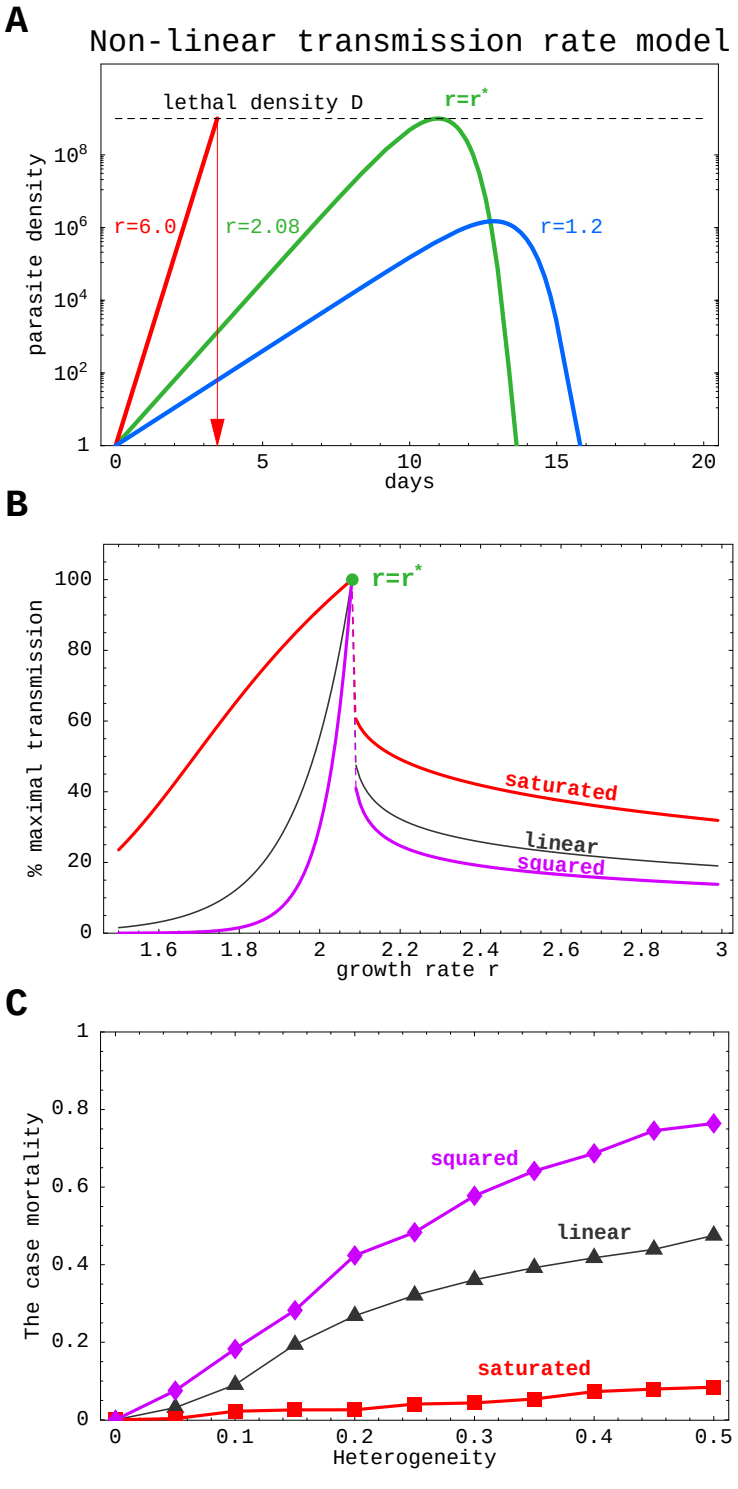

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Figure 3.2: Consequences of changing the transmission function (the function describing the rate of transmission as a function of the within-host density of the parasite). Linear function: $\zeta(P) = P$, saturating function: $\zeta(P) = \frac{P}{1+P/\theta}$, "square" function: $\zeta(P) = P^2$. Panel A shows that the within-host dynamics of parasites with different growth rates is not affected by the changes in the transmission function. Panel B shows the total transmission of parasites (normalized with respect to the maximum transmission) as a function of the growth rate of the parasite, for the different transmission functions. Panel C shows the virulence (case mortality) of the optimal parasite as a function of the degree of host heterogeneity for the different transmission functions. Heterogeneity is described by the coefficient of variation ($CV = \sqrt{variance}/mean$) in the gamma distribution chosen for the lethal density, D. Parameters are the same as in Figure 3.1 and $\theta = 10^7$.

3.4 Changing the term for pathogenesis

In the basic model pathogenesis was introduced by having a lethal parasite density, D. We assumed that the parasite kills infected hosts when it reaches this density within the host. There are many other mechanisms by which the parasite can induce pathogenesis. In this section we consider the consequences of changing the term for parasite-induced pathogenesis from lethal density to resource depletion (Marsh and Snow, 1997). In the resource depletion model, we assume that the parasite consumes a resource within the host, and this can cause the infected host to die if the level of resource falls below a threshold value. We call these two models the lethal density model and the resource depletion model.

We use a chemostat-type model for the resource, R (Pirt, 1975). Resource is generated at the rate $dR(0)$, and has a background turnover rate d. We assume that the parasite's growth rate is dependent on the consumption of the resource at the per capita rate $rR/(c+R)$ (Monod, 1949), and conversion efficiency y. Pathogenesis is introduced by assuming

that the parasite kills the host if the resource density falls below some critical value $R_d < R(0)$. The terms for the immune response and transmission rate remain unchanged from the earlier model (i.e., Eqns. (3.4) and (3.5)). The rates of change in the density of resource, $R(t)$, and parasite, $P(t)$, are thus given by the equations:

$$\frac{dR(t)}{dt} = d(R(0) - R(t)) - y^{-1}\frac{rR(t)P(t)}{c + R(t)} \tag{3.8}$$

$$\frac{dP(t)}{dt} = \frac{rR(t)P(t)}{c + R(t)} - hX(t)P(t), \quad \text{if } R(t) > R_d, \tag{3.9}$$

$$P(t) = 0, \quad \text{if } R(t) \leq R_d. \tag{3.10}$$

For simplicity, we consider the case when the rate of the resource turnover is slow in comparison with the time scale of an acute infection (i.e., $d = 0$), and parasite growth is not resource limited (i.e., $R_d > c$). A more comprehensive discussion of the other possibilities is presented in Appendix 2.

In Figure 3.3A we show the dynamics of the infection for parasites with different growth rates in the resource depletion model. To facilitate comparisons we choose R_d such as the optimal growth rate in this model in the absence of host heterogeneity is the same as in the lethal density model $r = r^* = 2.08$. We see that the within-host dynamics of infection are not substantially changed when the mechanism of pathogenesis is changed from lethal density to resource depletion. More detailed analysis revealed that when the growth rate is slightly greater than the optimal value (i.e., $r = r^* + \epsilon$, where ϵ is small) the parasite depletes the resource when it has been almost cleared. Consequently, there is only a minimal loss of transmission due to killing the host (in comparison with the large cost of killing the host in the lethal density model described earlier). This is reflected in Figure 3.3B we show how the (normalized) total transmission, $l(r)$, depends on the growth rate, r, of the parasite. We see that the major change introduced by this model occurs when the growth rate, r, exceeds optimal growth rate, r^* (i.e., when $r > r^*$). In this regime the resource depletion model gives a more gradual decrease in the total transmission with increasing growth rate r than the earlier lethal density model.

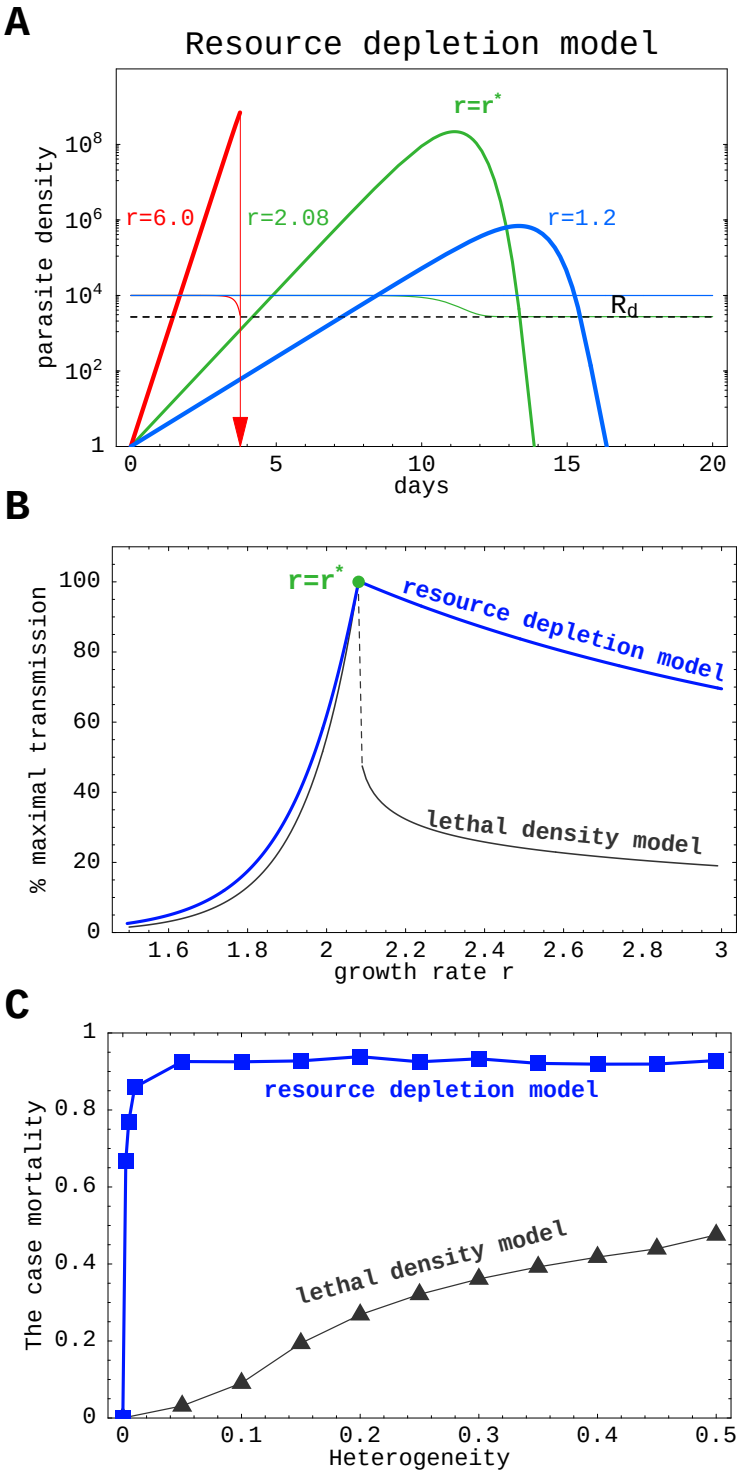

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Figure 3.3: Consequences of changing the mechanism of pathogenesis from a lethal density to resource depletion. Panel A shows the within-host dynamics of parasites (thick lines) and resource (thin lines) for parasites with different growth rates. Panel B shows the total transmission of parasites (normalized with respect to the maximum transmission) as a function of the growth rate of the parasite for the lethal density and resource depletion models. Panel C shows the virulence (case mortality) of the optimal parasite as a function of the degree of host heterogeneity for the lethal density and resource depletion models. Heterogeneity is described by the coefficient of variation ($CV = \sqrt{variance}/mean$) in the gamma distribution chosen for the lethal density, D, or minimum resource density, R_d. Parameters used for simulations are the same as in Figure 3.1 and $R(0) = 10^4$, $c = 10^3$, $y = 10^5$, $R_d = 2.7 \cdot 10^3$, $d = 0$.

In Figure 3.3C we show how virulence of the optimal parasite (measured by the case mortality) changes with the degree of host heterogeneity (introduced in the lethal density D in the lethal density model and the critical resource density R_d in the resource depletion model). In the lethal density model, the case mortality increases slowly with increasing heterogeneity. In contrast, in the resource depletion model the case mortality increases very rapidly with increasing heterogeneity and then saturates. These results suggest that changing the mechanism of pathogenesis can substantially alter the virulence of an evolved parasite.

3.5 Discussion

There is a large body of literature on the evolution of parasite virulence and the reader is directed to excellent reviews on this subject (e.g., (Bull, 1994; Frank, 1996; Ebert, 1999)). The hypotheses for the observed virulence of parasites include the following. (i)

The virulence of parasites may be coincidental (i.e., unrelated to their fitness) (Levin and Bull, 1994). (ii) The virulence of parasites may arise as a consequence of the within-host competition resulting from high mutation rates, co- or superinfection (Frank, 1992; Bonhoeffer and Nowak, 1994b; Nowak and May, 1994; May and Nowak, 1995; van Baalen and Sabelis, 1995; Mosquera and Adler, 1998). (iii) The virulence of parasites may arise as a consequence of their evolutionary adaptation to maximize their total transmission in the host population (Anderson and May, 1982). In this latter view, known as the adaptive framework, virulence arises as a consequence of the trade-offs between the transmission rate, host recovery rate, and virulence of the infection caused by the parasite. We also note that, in general, the evolution of the host in response to the parasite will result in the reduction of parasite virulence, possibly leading to a co-evolutionary arms-race between parasite and host (Gilchrist and Sasaki, 2002).

We have focused on the adaptive framework. Within this framework, a number of factors, including the route of parasite transmission (Ewald, 1983), host resistance (Gandon et al., 2001a), and the interaction of the parasite and the host immune response (Antia et al., 1994), have been proposed to affect the optimal level of virulence.

In this paper, we used a model of the within-host dynamics of microparasites that cause acute infections in vertebrate hosts. Using this model we explored how changes in the rate of parasite transmission from infected hosts and mechanisms of parasite-induced pathogenesis affect the level of virulence to which a parasite evolves. Our results suggest that changing the transmission rate or mechanism of pathogenesis can result in dramatic changes in this optimal level of virulence of the parasite.

What are the implications of our results for understanding the evolution of virulence of parasites? Our major result is that predicting the optimal level of virulence of a parasite will require a detailed quantitative understanding of the interaction of the parasite and its host. These could include the mode of parasite transmission (direct, indirect, vector-borne, etc.), how the rate of transmission depends on the parasite density, the interactions between the parasite and non-specific immunity (Antia and Koella, 1994; Pilyugin and An-

tia, 2000), the mechanisms of generation of specific immune responses (Kaech et al., 2002; Antia et al., 2003), intra-host competition (Leung and Forbes, 1998), different mechanisms of parasite-induced pathogenesis (target cell vs. resource depletion, toxin production, etc), and host heterogeneity (Ebert and Hamilton, 1996; Regoes et al., 2000; Ganusov et al., 2002). Furthermore, our results strongly suggest that in the absence of such an understanding it may be difficult to predict the extent to which changes in a single parameter will change the optimal level of virulence of a parasite.

3.6 Appendix 1: Calculating the average total transmission and case mortality

For a parasite with the growth rate r we calculate two parameters: (1) the average total transmission of the parasite in a heterogeneous host population, $L(r)$, representing fitness of the parasite, and (2) the case mortality caused by the parasite, $M(r)$, representing virulence of the parasite. To describe host heterogeneity, we use a gamma distribution of the lethal density D. Then $f(D)\,dD$ is a probability of choosing a host with the lethal density in the range $(D, D + dD)$.

The average total transmission of a parasite with the growth rate r in a heterogeneous host population characterized by the probability distribution $f(D)$ is

$$L(r) = \int_0^\infty l(r, D) f(D)\, dD. \tag{3.11}$$

where $l(r, D)$ is the total transmission of a parasite with the growth rate r infecting a host with the lethal density D ($l(r, D)$ is given in Eq. (3.5)). We numerically calculate the integral in Eq. (3.11) by evaluating $l(r, D) \cdot f(D)$ for every D at fixed r.

We find the optimal growth rate of the parasite r_{opt} at which the average total transmission $L(r)$ reaches its maximum using a "finding a minimum" algorithm (*brent*) from the Numerical Recipes (http://www.library.cornell.edu/nr/bookcpdf/c10-2.pdf).

The case mortality is the probability that a randomly chosen host will die following infection. To estimate the case mortality of a parasite with the growth rate r, we first calculate the maximum density, $P_{max}(r)$, that the parasite can reach during the acute infection (assuming no host mortality, i.e., $D = \infty$). The case mortality is simply the fraction of hosts which have their lethal densities below $P_{max}(r)$:

$$M(r) = \int_0^{P_{max}(r)} f(D)dD. \tag{3.12}$$

3.7 Appendix 2: The evolution of extra-cellular microparasites depleting host resources during the acute infection

In the main text we restricted our analysis of the resource depletion model to a particular case when the rate of the resource turnover is zero, i.e., $d = 0$. Here we investigate how the rate of the resource turnover, d, affects the evolution of extracellular parasites that kill their hosts by depleting the host resource during acute infection.

We find that there are two critical parameters in the model which determine the within-host dynamics of parasites. These are the rate of the resource turnover, d, and the critical resource density, R_d. The last parameter affects the dynamics of parasites differently depending on whether $R_d \gg c$ or $R_d \ll c$, where c is the half-saturation constant for the growth rate of the parasite (see eq. (3.9)). In all cases the parasite that depletes the host resource until the density R_d achieves maximal total transmission.

When $R_d \gg c$, the parasite grows approximately exponentially at the maximum rate r until it either kills the host or is controlled and cleared by the immune response. As the rate of the resource turnover, d, increases, we find that (1) the optimal growth rate of the parasite increases at all else being equal, and (2) the minimal density of the resource during the acute infection becomes inversely correlated with the peak of parasitemia (Figure

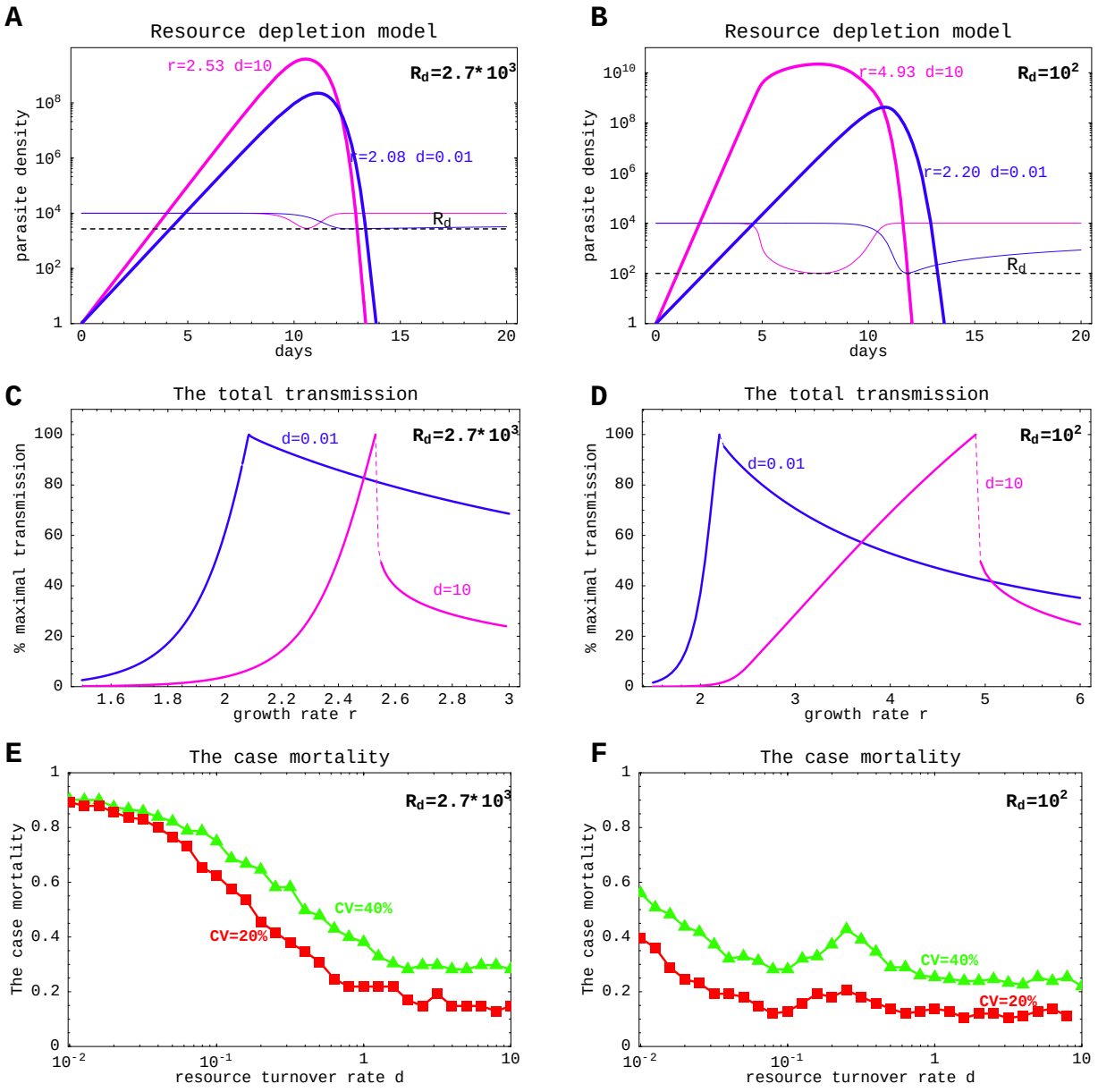

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Figure 3.4: The evolution of extra-cellular microparasites according to the resource depletion model in a heterogeneous host population. Panels A & B show the within-host dynamics of optimal parasites at different rates of resource turnover d when the minimal resource density is high (A, $R_d = 2.7 \cdot 10^3 \gg c$) and low (B, $R_d = 10^2 \ll c$). Thick lines – parasites, thin lines – resources, a dashed horizontal line denotes the minimal resource density R_d. Panels C & D show the total transmission of parasites (normalized with respect to the maximum transmission) over the course of acute infection as a function of the growth rate at high (C) and low (D) minimal resource density. The rate of the resource turnover is marked. Panels E & F show virulence (case mortality) of the optimal parasite as a function of the resource turnover rate when minimal resource density R_d is high (E) or low (F). Heterogeneity is described by the coefficient of variation ($CV = \sqrt{variance}/mean$) in the by the gamma distribution chosen for the minimal resource density R_d. Note that higher levels of host heterogeneity lead to higher case mortality in accordance with previous results. Parameters are the same as in Figure 3.3 with $d > 0$.

3.4A). Both changes are intuitively obvious. As the rate of resource turnover increases, the minimal resource density during the infection increases; therefore, in order for the parasite to deplete the resource to R_d, it must increase its growth rate. At high rates of resource turnover the resource becomes a "fast" variable, changes in which are rapidly adjusted to the changes in the parasite density.

The parasites with the growth rate just infinitesimally higher than the optimal will pay much higher cost of killing the host (i.e., loss in the total transmission) when the rate of resource turnover is high (Figure 3.4C). As a consequence we find that the optimal level of virulence, measured by the case mortality, monotonically decreases with the increasing rate of the resource turnover for a given level of heterogeneity in R_d (Figure 3.4E).

When $R_d \ll c$ the within-host dynamics of the parasite and resource remain similar to the previous case except the case when the resource turnover is high. When d is

high, parasite density saturates as the resource density falls below c (Figure 3.4B). The growth rate at which the total transmission of the parasite is maximal increases with the increasing rate of resource turnover. The cost of killing the host is also high when the resource turnover is high (Figure 3.4D).

However, in contrast with the previous case, the optimal level of virulence of the parasite is not strictly a decreasing function of the turnover rate d (Figure 3.4F); even though the general trend is observed, at some intermediate turnover rates ($d = 10^{-1} - 5 \cdot 10^{-1}$) the case mortality increases with the increasing turnover rate. There are two factors which lead to changes in the case mortality. First, as the rate of resource turnover increases, the case mortality of a parasite with the fixed growth rate r decreases because the parasite depletes less resource (see Figure 3.4A–B). On the other hand, an increase in the growth rate at fixed d will lead to a higher case morality because the parasite depletes the resource to a lower density. As the rate of resource turnover increases, the optimal growth rate increases, and as the result, the case mortality may change in either way (decrease or increase), depending on which of the changes (turnover rate vs. growth rate) has a greater impact. It seems that the general trend is nevertheless robust — when the resource turnover is high, the optimal level of virulence of the parasite is low and *vice versa*.

These changes in the optimal level of parasite virulence with changes in the rate of resource turnover are not specific to heterogeneity in R_d; the same trend is observed when heterogeneity is introduced into the parameter k (see Eq. (3.4) and Figure 3.5). Interestingly, with heterogeneity in k the case mortality decreases monotonically with the rate of resource turnover d even at $R_d \ll c$.

Explicit trade-offs. Previously we assumed that parasites evolve their growth rate r and that changes in the growth rate do not affect other parameters. However, we might expect that parasites that grow faster may utilize the resource with lower efficiency. What would happen if such a trade-off is explicitly introduced into the resource depletion model?

We tested two different types of the trade-off: linear ($y(r) = y_0(10 - r)$) and hyperbolic

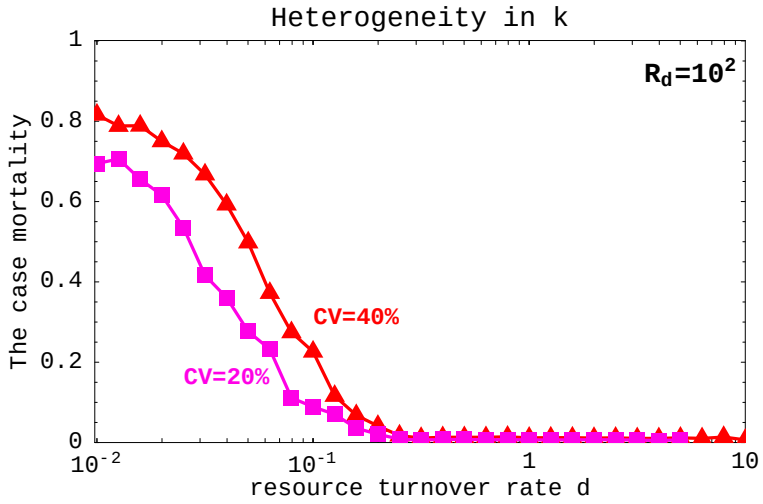

Figure 3.5: Virulence (case mortality) of the optimal parasite as a function of the resource turnover rate when heterogeneity is described by a gamma distribution of k. Heterogeneity is decribed by the coefficient of variation ($CV = \sqrt{variance}/mean$). Parameters are the same as in Figure 3.3 except $R_d = 10^2$.

($y(r) = y_0/r$), and the qualitative trend seems to be independent of the particular trade-off type (not shown). The difference between the maximum total transmission (at $r = r^*$) and total transmissions of parasites with smaller growth rate ($r < r^*$) is lower when there is trade-off (Figure 3.6A; the difference is obvious when $d = 10$ but is too small to be seen for $d = 0.01$). Parasites with higher growth rates ($r > r^*$) have a lower total transmission when there is a trade-off. Introducing heterogeneity in R_d we find that the trade-off between efficiency of resource consumption and the growth rate generally leads to selection of less virulent parasites (Figure 3.6B); the exact nature of the trade-off (for example, linear vs. hyperbolic) also influences the exact level of virulence parasites evolve (not shown).

3.8 Publication status

Ganusov, V.V., and Antia, R. (2003) Trade-offs and the evolution of virulence of microparasites: do details matter? *Theor Pop Biol* 64: 211–220

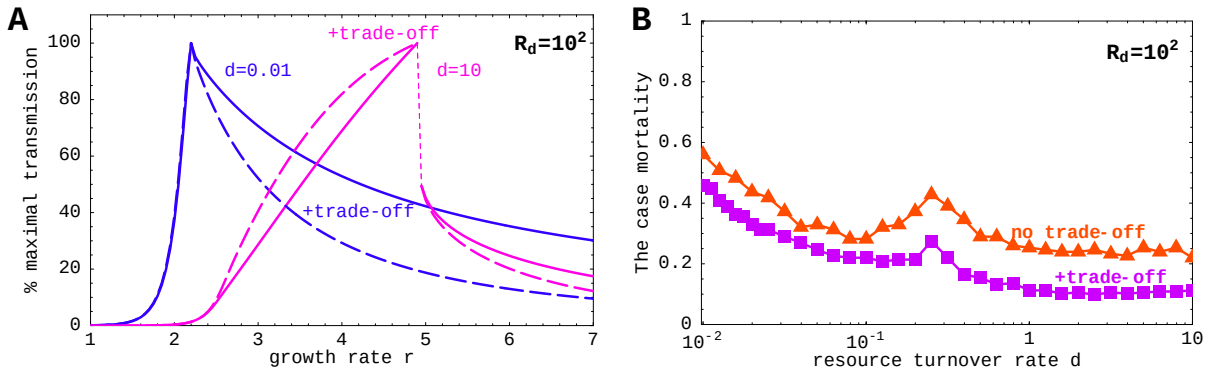

Figure 3.6: The evolution of extra-cellular microparasites when there is a constrain (trade-off) on the efficiency of resource consumption y and the growth rate r. Panel A shows the total transmission of parasites (normalized with respect to the maximum transmission) over the course of acute infection as a function of the growth rate in the absence (solid lines) and presence (dashed lines) of the trade-off. The rate of the resource turnover is marked. Trade-off functions used are $y = 4.9 \cdot 10^5/r$ for $d = 10$ and $y = 2.2 \cdot 10^5/r$ for $d = 0.01$ (parameters are chosen to provide peaks of the total transmission at the same growth rates at two rates of resource turnover). Panel B shows virulence (case mortality) of the optimal parasite as a function of the resource turnover rate in the presence or absence of the trade-off. Heterogeneity is decribed by a gamma distribution in the minimal resource density R_d ($CV = 40\%$, $\overline{R_d} = 10^2$). For a linear trade-off ($y = 10^5(10 - r)$) similar results were obtained (not shown). Parameters are the same as in Figure 3.4.

Chapter 4

The role of the CTL response and virus cytopathogenecity in the virus decline during antiviral therapy

Abstract

Although it is clear that HIV can lyse HIV-infected CD4 T cells, it is still controversial whether the depletion of CD4 T cells seen in HIV-infected patients after years of asymptomatic disease is due to direct cytopathic effects of the virus or is mediated by the immune response. Assuming that the initial decline in viremia during active antiretroviral therapy (HAART) is due to death of cells, productively infected with HIV, we investigate how the rate of the virus decline is affected by the efficiency of the CTL response. We find that whether the stronger immune response causes more rapid virus decline depends critically on how the virus is controlled by the CTL response (lytic vs. nonlytic mechanisms). Moreover, the variation in the efficiency of the immune response does not always cause variation in the rate of the virus decline (and therefore, in the death rate of infected cells), implying that the constancy of the virus decline rate measured in different patients does not necessarily indicate that the virus is cytopathic. The potential problems associated with the model and undertaken approach are also discussed.

4.1 Introduction

It is still unknown whether HIV is cytopathic *in vivo*, i.e., whether the depletion of CD4 T cells serving as a primary target for HIV occurs mainly due to killing of virus-infected cells by the virus itself (Perelson, 2002). An alternative hypothesis is that the virus is relatively non-cytopathic *in vivo* and the majority of cell death occurs due to immune response mediated destruction of virus-infected cells (Klenerman and Zinkernagel, 1997). Both hypotheses are supported by indirect evidence at least *in vitro* but conclusive evidence of the relative role of these two processes *in vivo* is still lacking. Understanding of why virus-infected cells die may help design better therapies of treatment of the disease and other strategies for slowing or stopping the disease progression.

HIV is clearly cytopathic *in vitro*: in culture cells infected with the virus die more rapidly than uninfected controls (Levy, 1998); it is likely that both the direct killing of infected cells by the virus and indirect killing of bystander uninfected cells contribute to this effect (McCune, 2001). Similarly, cytotoxic T lymphocytes (CTLs) can lyse HIV-infected cells in a standard ^{51}Cr release assay (Klenerman et al., 1996; Yang et al., 1996). It has been difficult, however, to evaluate the relative role of these two processes in the life-span of productively infected CD4 T cells. Using drugs preventing virus replication, it has been estimated that such cells live on average 1 day with little variation between individuals with different CD4 T cell counts (Wei et al., 1995; Ho et al., 1995; Perelson et al., 1996; Perelson, 2002). This observation has led to a suggestion that HIV must be highly cytopathic *in vivo* killing CD4 T cells in approximately 1 day. Otherwise, if the life-span of infected cells were determined by the immune response, one would expect much greater variation in the life-span of infected cells between different individuals with different immune responses (Nowak et al., 1996). This verbal argument is further supported by mathematical modeling suggesting that the life-span of cells infected with non-cytopathic viruses should vary to a greater extend when measured in patients with different immune responses than for highly cytopathic viruses (Klenerman et al., 1996).

The main problem with such verbal logic and supporting mathematical modeling, however, is that immune responses have not been incorporated explicitly into the models. This makes it difficult to evaluate which parameters and processes are important in such a conclusion. Recently, Arnaout et al. (2000) analyzing the dynamics of virus-infected cells and CTLs during HAART have found that the life-span of virus-infected cells may be independent of the efficiency at which CTLs lyse virus-infected cells. Here we extend their analysis and reanalyze factors that may lead to variation in the life-span of productively infected T cells between different patients.

We do that by assuming that the initial decline in viremia during HAART is due to death of cells, productively infected with the virus[1]; therefore, the rate of virus decline is proportional to the death rate of virus-infected cells. Given that we further focus on whether the efficiency of the CTL response may affect the decline rate of the virus during HAART (and therefore, the life-span of infected cells).

We find that whether the immune response affects the rate of virus decline depends critically on how the virus is controlled by CTLs. If the virus is controlled only either by killing virus-infected cells or by reducing rate of virus replication (through a release of antiviral cytokines and chemokines), then variation in the efficiency of the immune response does not lead to variation in the life-span of infected cells. This is simply because the efficiency of the immune response (that is the rate at which a given effector cell kills a virus-infected cell) is compensated by the number of HIV-specific CTLs. This in turn happens because to balance the virus replication and virus death at the steady state, higher CTL efficiency requires less CTLs (and *vice versa*). The conclusion holds even if both mechanisms are acting together but only if they are interdependent (i.e., the efficiency of killing of infected cells is correlated with the efficiency of production of effector cytokines and chemokines). However, if both mechanisms (killing and releasing cytokines) are independent, then the variation in the efficiency of either effector mechanism will lead

[1]This follows from the fact that prior to HAART the majority of the virus is produced by productively infected cells (Perelson et al., 1996).

to variation in the measured life-span of infected cells.

Thus from the analysis follows that the variation in the life-span of productively infected cells (or the absence of thereof) is indicative of the cytopathogenecity of a virus only at a restricted set of assumptions and it is not yet clear whether those assumptions are fulfilled for HIV.

The paper is structured as follows. In Section 4.2 we shortly describe the conventional results on the virus dynamics during HAART and its interpretation. In Section 4.3 we analyze a simple mathematical model describing the dynamics of virus-infected cells and the immune response during HAART. In Section 4.4 we discuss the implications of main results of the analysis and possible problems associated with the model and undertaken approach.

4.2 Virus dynamics during HAART

A striking property of HIV infection is that during the asymptomatic period of several years viremia (viral load in blood) remains approximately constant (Levy, 1998, p. 317-321). The constant viremia, however, results from a balancing production and clearance of the virus with more than 95% of the virus being turned over each day. This dynamic property of HIV infection has been discovered using drugs that suppress virus replication, namely reverse transcriptase and protease inhibitors (Wei et al., 1995; Ho et al., 1995; Perelson, 2002).

When the drugs are administered, viral load declines in several phases (see Figure 4.1). First, there is an initial delay in the virus decline due to pharmacological and virus life-cycle delays (Perelson et al., 1996; Herz et al., 1996; Nelson et al., 2001; Lloyd, 2001). Then, during the first phase, viral load declines rapidly with the half-life time constant ~ 1 *day*. The overall drop in viremia during this phase varies between patients whereas the rate at which viral load declines is strikingly independent of a patient's CD4 T cell count (a representative of immune system health). Approximately one week later, there

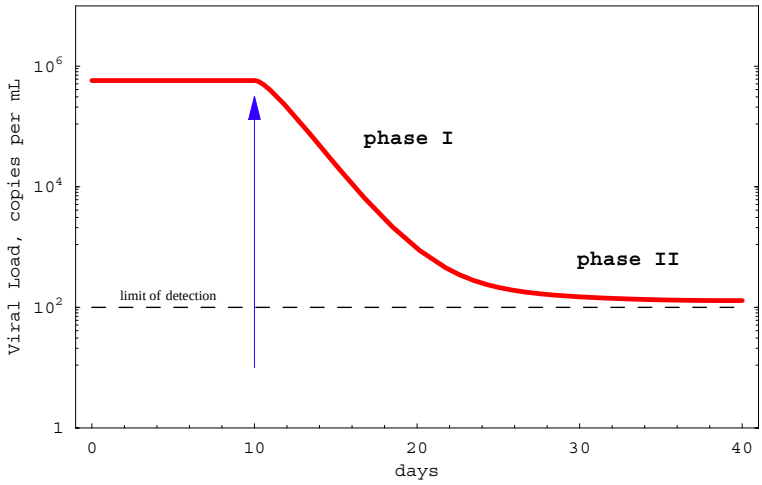

Figure 4.1: The dynamics of HIV during highly active antiretroviral therapy. It is assumed that before day 10 the viral load is constant (asymptomatic phase) and then at day 10 the drugs suppressing virus replication are administered. During the phase I viral load declines rapidly with the half-life time $t_{1/2} = 0.8\ day$ (this phase corresponds to the death of productively infected cells). During the phase II, viral load declines more slowly with the half-life time $t_{1/2} = 30.7\ days$ (representing the half-life of latently infected cells).

is a second slower phase during which viral load decreases with the average half-life time of $15 - 30\ days$ (Perelson et al., 1997). It has been proposed that the virus decline during the first phase represents the death of productively infected CD4 T cells whereas during the second phase cells of other types (such as macrophages or latently infected CD4 T cells) are being eliminated (Perelson et al., 1997). Although as it should be noted other interpretations for the virus dynamics during HAART have also been suggested (Ferguson et al., 1999; Grossman et al., 1999; Arnaout et al., 2000; Hlavacek et al., 2000; Muller et al., 2001), in the following analysis we nevertheless assume that the virus decline in the phase I (i.e., the first week of therapy) is due to death of productively infected cells. And more specificly, we ask how the CTL response may affect the initial rate of virus decline and therefore, the death rate of virus-infected cells.

4.3 The model

Although it is not known what controls HIV during the asymptomatic phase CTLs are thought to play some role in restricting virus replication (Koenig et al., 1995; Evans et al., 1999; Jin et al., 1999; Metzner et al., 2000). There is a number of ways of how CTLs may do that. First, HIV-specific CTLs can lyse virus-infected cells. Second, CTLs release chemokines such as β-chemokines (MIP-1α, MIP-1β, RANTES) that can prevent infection of new cells (Cocchi et al., 1995). Finally, CTLs release cytokines such as INF-γ that can suppress the rate of virus production by virus-infected cells (Yang et al., 1997; Guidotti and Chisari, 2001).

Since the last two mechanisms effectively reduce the rate of virus infection of uninfected cells, we assume that CTLs affect virus replication in two major ways: by killing virus-infected cells (lytic mechanisms) and by reducing the rate of virus replication (nonlytic mechanisms). Since free HIV particles are short-lived (Perelson et al., 1996; Ramratnam et al., 1999; Zhang et al., 1999) the virus concentration is approximately proportional to the density of virus-infected cells. We also assume that the changes in the total number of uninfected cells are small during the first week of HAART.

The remaining details of the model are as follows. Infected cells Y have per capita growth rate r and the death rate due to viral cytopathogenecity α. The presence of the immune response reduces the replication rate of the virus to $r/(1+aZ)$ where Z is the number of CTLs controlling the virus and a is the efficiency of CTLs in reducing the virus replication rate. Infected cell are also killed by CTLs at per capita rate h. Stimulation of the immune response by the virus is described by a function $f(Y,Z)$. The mathematical model then becomes (see Appendix[2] for a more general model):

$$\dot{Y} = \frac{rY}{1+aZ} - \alpha Y - hYZ, \tag{4.1}$$

$$\dot{Z} = f(Y,Z). \tag{4.2}$$

[2]Available from the Royal Society website.

During the chronic (asymptomatic) phase viral load (and most likely, the number of T cells productively infected with HIV) is approximately constant; therefore, the steady states of the model (4.1)–(4.2) should describe the asymptomatic phase of infection. These steady states are:

$$\frac{r}{1+a\overline{Z}} = \alpha + h\overline{Z}, \qquad f(\overline{Y},\overline{Z}) = 0. \tag{4.3}$$

Administration of drugs preventing the production of infective virions (protease inhibitors) and *de novo* infection (reverse transcriptase inhibitors) reduces the rate of virus replication to the value $(1-\rho)r$ with the drug efficiency ρ. Although the antiviral drugs may interfere with the proliferation of T cells, the mechanism of this phenomenon is not understood (Levy, 1998, pp. 354-356); for the simplicity, we assume that the drugs do not affect the immune response directly. However, the decline in viral load can indirectly affect the immune response. It has been shown that the number of HIV-specific CD8 T cells (measured by MHC class I + specific peptide tetramers) declines during HAART (Ogg et al., 1999; Casazza et al., 2001). It is not clear, however, whether the number of functional CD8 T cells changes in accord. In the model we assume that the number of CTLs changes from a steady state value given in eq. (4.3) with a per capita rate δ (which can also be negative):

$$\dot{Y} = (1-\rho)\frac{rY}{1+aZ} - \alpha Y - hYZ, \tag{4.4}$$

$$\dot{Z} = f(Y,Z) \approx -\delta Z. \tag{4.5}$$

Since we do not know how CTLs control HIV, we consider three different scenarios: when CTLs are only able to lyse infected cells (i.e., at $h > 0$ and $a = 0$), when CTLs can only affect virus replication (i.e., $h = 0$ and $a > 0$), and when both mechanisms are used by CTLs and are of the same order of magnitude.

CTLs only kill ($a = 0$)

Initially, we assume that the rate of CTL decline during HAART is small, i.e. $\delta \ll r$ (Ogg et al., 1999; Casazza et al., 2001). Then the changes in the number of CTLs during initial days of HAART are also small; therefore, we can replace the number of CTLs $Z(t)$ by its stationary value $\overline{Z} = (r - \alpha)/h$ given in eq. (4.3) at $a = 0$. Then, the dynamics of virus-infected cells simply become:

$$\dot{Y} = (1 - \rho)rY - \alpha Y - hYZ \approx (1 - \rho)rY - \alpha Y - hY\overline{Z} = -\rho rY, \tag{4.6}$$

We find that the rate at which the number of infected cells declines is independent of the efficiency of the immune response h. This in turn happens because lower h require more CTLs to control the virus (see eq. (4.3)) and vice versa. A similar result has been obtained by Arnout et al. (2000). Moreover, the rate of virus decline is the product of the rate of virus replication r and the drug efficacy ρ. Thus, the variation in the rate of virus decline during the first phase can be simply because of the drug efficacy and growth rate variation between different patients (Bonhoeffer et al., 1997).

If the rate of change of HIV-specific CTLs is not small ($\delta \sim r$), then the virus decline is not strictly exponential (see Figure 4.2). Despite this fact, the initial decline rate is still independent of the strength of the immune response h.

CTLs only reduce the virus replication rate ($h = 0$)

Similarly to the previous case, replacing $Z(t)$ with its stationary value $\overline{Z} = (r - \alpha)/(\alpha a)$ at $h = 0$ the dynamics of virus-infected cells become:

$$\dot{Y} = (1 - \rho)\frac{rY}{1 + aZ} - \alpha Y \approx (1 - \rho)\frac{rY}{1 + a\overline{Z}} - \alpha Y = -\rho\alpha Y, \tag{4.7}$$

Here again the rate of decline is independent of the strength of the immune response (now a), but now it is a product of the death rate of infected cells α and the drug efficacy ρ.

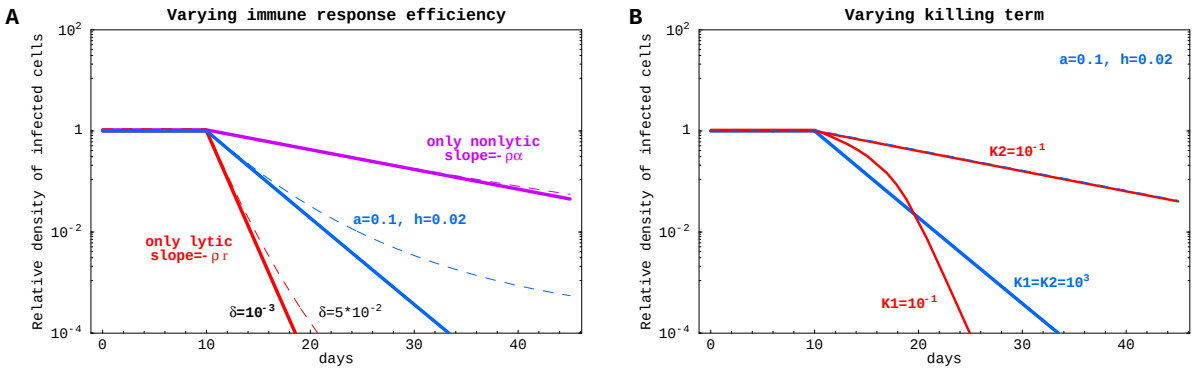

Figure 4.2: The influence of the immune response on the virus dynamics during HAART. Panel A: the virus decline when the efficiency of the immune response varies. Three parameter combinations are shown: $a = 0.1,\ h = 0$ (only nonlytic mechanisms), $a = 0.1\ h = 0.02$ (both lytic and nonlytic mechanisms), and $a = 0,\ h = 0.02$ (only lytic mechanisms). Two rates of CTL decline are considered: $\delta = 10^{-3}$ (solid lines) and $\delta = 5 \cdot 10^{-2}$ (dashed lines). Panel B: the virus decline when saturation in the killing rate is considered (i.e., in the equation (4.1) the term hYZ is replaced with $hYZ/(1 + Y/K_1 + Z/K_2)$). Saturation in the killing rate on the density of infected cells leads to non-exponential virus decline ($K_1 = 10^{-1}$, $K_2 = 10^3$). Saturation in killing rate on the density of CTLs reduces the decline rate ($K_1 = 10^3$, $K_2 = 10^{-1}$) even when $K_1 = 10^{-1}$ (dashed line). Other parameters: $r = 1.2$, $\alpha = 0.1$, $\rho = 0.9$.

CTLs kill and affect the virus replication rate ($h > 0$ and $a > 0$)

Applying the same technique as before at $\delta \ll r$, we find:

$$\dot{Y} = (1-\rho)\frac{rY}{1+aZ} - \alpha Y - hYZ \approx (1-\rho)\frac{rY}{1+a\overline{Z}} - \alpha Y - hY\overline{Z} = -\rho\frac{rY}{1+a\overline{Z}}, \quad (4.8)$$

where $\overline{Z} = \left(\sqrt{(\alpha a - h)^2 + 4rha} - (\alpha a + h)\right)/(2ha)$.

In this case, the rate of virus decline depends upon both parameters a and h, characterizing the strength of the immune response. However, equation (4.8) can be rewritten assuming $h = c \cdot a$, where c is a constant:

$$\dot{Y} \approx -\frac{r\rho}{1 + \left(\sqrt{(\alpha - c)^2 + 4rc} - \alpha - c\right)/(2c)}Y. \quad (4.9)$$

from which follows that the rate of virus decline depends only on the ratio of effector cell efficiencies and not their absolute values. Importantly, if a and h vary independently between different individuals leading to $c = h/a$ varying from 0 to ∞, then the rate of virus decline will also vary from $\rho\alpha$ to ρr (Figure 4.2:A). A similar conclusion holds if both effector mechanisms are correlated (i.e., $h = c \cdot a$) but with a constant c different between different patients. If either of these assumptions were true for HIV, the observed small variation in the virus decline rate would imply that $r \approx \alpha$ or almost all death of virus-infected cells occurs because of virus cytopathogenecity. Unfortunately, as far as we are aware it is not known whether there is a correlation between two effector mechanisms for HIV-specific CTLs.

Thus, we find that whether the strength of the immune response controlling the virus affects the measured life-span of virus-infected cells depends critically on how the virus is controlled and whether effector mechanisms by which the virus is controlled are correlated.

4.4 Discussion

In this paper we analyzed how the immune response, or more precisely, the variation in the efficiency of the immune response, affects the rate of virus decline during the first week of HAART. We found that the result critically depends on how the immune response (CTLs in particular) control the virus. For instance, if CTLs only kill virus-infected cells or only reduce the rate of virus replication (by releasing antiviral cytokines and chemokines), then the variation in the killing/suppressing efficiently does not affect the rate of virus decline. This is simply because at the steady state to control the virus, the efficiency of the immune response (per effector cell) must be compensated by the number of the virus-specific CTLs (see also Bucy (1999)). A similar conclusion holds even when both mechanisms are acting and are correlated (i.e., $h = \text{const} \cdot a$), with a coefficient of proportionality being constant between different individuals. However, if the killing efficiency is independent of the suppressing efficiency (i.e., $h \neq \text{const} \cdot a$) then independent variation in h and a leads to variation in the rate of virus decline that is bounded by ρr and $\rho\alpha$. Unfortunately, it is not clear if the release of cytokines and chemokines is correlated with the release of perforin and granzymes for antigen-specific CD8 T cells. Although both processes are triggered when there is an appropriate ligand for TCR, the conclusive evidence on the linkage between two effector mechanisms as far as we are aware is still lacking.

It is also important to note that although the model (4.1)–(4.2) does not include the dynamics of the free virus and uninfected cells, the major conclusion holds in a more general model (see Appendix). However, we find that functional changes in the killing term such as to include saturation in the killing rate as the number of CTLs or infected cells increases may affect the rate of virus decline (Figure 4.2:B). It is not clear, however, whether saturation in the killing rate occurs *in vivo*. On the other hand, the functional form of how CTLs reduce replication rate of the virus does not affect the major result (not shown).

Fortunately, it is possible to test the presented mathematical model. The model predicts that the rate of virus decline during antiviral therapy should be independent of the number of functional CTLs, specific to the virus, existed prior to treatment (see, for example, eqns. (4.6) and (4.7)). For HIV infection, both viral load and the CTL number may be accurately estimated (Piatak et al., 1993; Sun et al., 2003). If, however, the opposite is found (i.e., the rate of decline *is* correlated with the number of functional CTLs specific to the virus) this would imply that (1) both lytic and nonlytic mechanisms are involved in controlling the virus, and (2) either the effector mechanisms are uncorrelated or correlation is different in different patients (i.e., constant c varies between different patients).

It is also necessary to emphasize that despite the relative robustness of the model predictions, there are several potential problems associated with the model as well as with the undertaken approach.

1. The model assumes that the initial virus decline is due to the death of cells, productively infected with the virus. In HIV infection, however, the virus decline may be affected by the release of the virus from lymphoid tissues into blood and in some circumstances may not represent the death of infected cells (Hlavacek et al., 2000; Muller et al., 2001).

2. The key assumption of the model is that the virus and the immune response are at the steady state prior to drug administration. Although it is most likely correct for the total population sizes of the virus and HIV-specific CTLs, there might be a very dynamical change in clone composition in both populations with new viral variants arising and new CD8 T cell responses generated, i.e., the population structure of the virus and CTLs may not be at the equilibrium.

3. The model in its simplest form predicts that the number of virus-specific CTLs is determined by the parameters r, α, h, and a and as a consequence is independent of viral load (see eq. (4.3)). This seems to contrast to experimental observation where negative, positive, or no correlation between two quantities depending on the

viral gene has been reported (Novitsky et al., 2003, and references therein). Since underlying mechanisms of such a relationship have not been elucidated, we did not investigate how the model can be modified to include these observations even though such attempts have been made (Wodarz et al., 2001).

The analysis conducted in this paper in no regard should be considered as a providing with the answer to whether HIV is cytopathic or not. Rather we tried to demonstrate that in order to understand why a given virus is cytopathic a simple analysis or common logic may simply be misleading. The dynamics of HIV and other infections such as HBV and HCV during antiviral therapy are complex with the immune response most likely playing an important role. Hopefully, future research will shed some light on whether the immune response efficiency affects the rate of virus decline during HAART as well as on whether HIV is controlled by the immune response during the asymptomatic phase and how such control if it exists is managed.

4.5 Appendix: the virus dynamics in a more general model

A more general model of the virus dynamics should include the dynamics of uninfected target cells (X), infected cells (Y), the free virus (V), and the immune response (Z):

$$\begin{aligned} \dot{X} &= \lambda - dX - \frac{\beta XV}{1+a_1 Z}, \\ \dot{Y} &= \frac{\beta XV}{1+a_1 Z} - \alpha Y - hYZ, \\ \dot{V} &= \frac{kY}{1+a_2 Z} - uV, \\ \dot{Z} &= f(V,Y,Z). \end{aligned} \tag{4.10}$$

In this model CTLs can affect virus replication via three different mechanisms: secretion of chemokines and prevention of infection of uninfected cells by the virus (a_1), release

of cytokines such as INF-γ to reduce the virus burst size (a_2) and direct killing of virus infected cells through perforin-granzyme and Fas-FasL pathways (h). The independence of the rate of virus decline during treatment of the CTL efficiency (h, a_1 and a_2) is achieved only when all the parameters are correlated (i.e., $h/a_1 = \text{const}_1$ and $h/a_2 = \text{const}_2$). A particular case of the model dynamics when $a_2 = 0$ is shown in Figure 4.3.

The simplified model (4.1)–(4.2) given in the main text can be obtained using the following approximations. First, because the free virus is short lived (Perelson et al., 1996; Ramratnam et al., 1999; Zhang et al., 1999), i.e. $u \gg 1$, we can use a quasi steady state assumption to obtain $V(t) \approx ku^{-1}Y(t)/(1 + a_2Z(t))$. Assuming that the number of uninfected cells, $X(t)$, does not change significantly during first weeks of the therapy, we replace $X(t)$ with its stationary value $\overline{X}$[3]. Finally, assuming $a_2 = 0$ we arrive to the model (1)–(2) with $r = \beta\overline{X}k/u$ and $a = a_1$.

4.6 Publication status

Ganusov, V.V. (2003) The role of the CTL response and virus cytopathogenecity in the virus decline during antiviral therapy. *Proc Roy Soc Lond B* 270: 1513–18

[3]At some parameter combinations, however, $X(t)$ in the full model may dramatically increase during HAART (see Figure 4.3:A). Although a similar increase has been observed for the CD4+ T cell number in the blood, it most likely represents a redistribution of T cells from the lymphoid tissues into the blood (Pakker et al., 1998; Bucy et al., 1999).

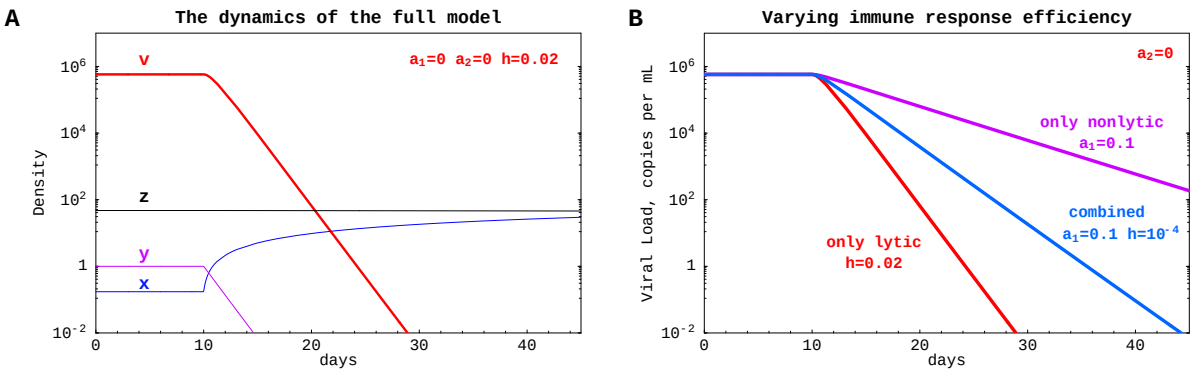

Figure 4.3: The dynamics of the virus during HAART according to the full model. Panel A: the dynamics of the virus V, uninfected cells X, virus-infected cells Y, and immune response Z. At time $t = 10$ HAART is started by stopping new infections ($\beta = 0$). Panel B: the virus decline when the efficiency of the immune response varies. Three parameter combinations are shown: $a_1 = 0.1$, $h = 10^{-5}$ (only nonlytic mechanisms), $a_1 = 0.1$ $h = 10^{-4}$ (both), and $a_1 = 0$, $h = 0.02$ (only lytic mechanisms). In all cases $a_2 = 0$. Other parameters: $\lambda = 1$, $d = 0.01$, $\alpha = 0.05$, $\beta = 10^{-5}$, $k = 10^6$, $u = 1.5$, $\delta = 10^{-3}$.

Chapter 5

The rescaling method for quantifying the turnover of cell populations

Abstract

The dynamic nature of immune responses requires the development of appropriate experimental and theoretical tools to quantitatively estimate the division and death rates which determine the turnover of immune cells. A number of papers have used experimental data from BrdU and *D*-glucose labels together with a simple random birth-death model to quantify the turnover of immune cells focusing on HIV/SIV infections (Mohri et al., 1998; Hellerstein et al., 1999; Bonhoeffer et al., 2000; Mohri et al., 2001). We show how uncertainties in the assumptions of the random birth-death model may lead to substantial errors in the parameters estimated. We then show how more accurate estimates can be obtained from the more recent CFSE data which allow to track the number of divisions each cell has undergone. Specifically, we: (i) describe a general stage-structured model of cell division where the probabilities of division and death are functions of time since the previous division; (ii) develop a rescaling method to identify invariant parameters (i.e. the ones that are independent OF the specific functions describing division and death); (iii) show how these invariant parameters can be estimated, and (iv) illustrate this technique by applying it to CFSE data taken from the literature.

5.1 Introduction

Understanding the quantitative aspects of cell turnover is a long standing theoretical problem. Specifically, we need a reliable analytical tool for estimating the rates of cell division and cell death that govern the rate of change in the total cell population. In immunology, for example, we would like to understand the mechanism of homeostatic regulation OF immune memory which results in a nearly constant cell population. Does such a population consist of quiescent cells or there is a balanced turnover of cells? If turnover occurs, how do cells progress through the cell cycle so that division and death processes balance each other?

Accurate quantification of the dynamics of antigen-specific B and T-lymphocytes *in vivo* has become possible recently (Slifka and Ahmed, 1998; Murali-Krishna et al., 1999). In addition, the development of CFSE[1] dye dilution experiments has allowed for accurate tracking of the number of divisions that a given cell has undergone following transfer *in vivo* (Weston and Parish, 1990; Lyons and Parish, 1994; Lyons, 2000). When cells are stained with CFSE, this fluoresecent dye is included into the cell cytoplasm. During the process of division, the CFSE dye is diluted approximately equally between the two daughter cells. For *in vivo* transfer experiments, the CFSE assay allows to accurately track up to 5-10 successive generations of cells. Consequently, the CFSE technique provides a significantly more accurate description of cell turnover compared to the earlier techniques for measuring cell turnover such as BrdU, thymidine and D-glucose labelling (Mohri et al., 1998; Hellerstein et al., 1999; Bonhoeffer et al., 2000; Mohri et al., 2001; Debacq et al., 2002). There has been a widespread interest in using CFSE data to quantify the birth and death processes underlying the dynamics of immune responses.

A common way to study the quantitative aspects of cell turnover is to formulate a specific model for cell division and death and fit this model to the data. This approach has two serious shortcomings. First, in the absence of a biologically validated model it

[1]Abbreviations used in this paper: CFSE, carboxyl fluorescein succimidyl ester; BrdU, bromodeoxyuridine; FLM, fraction of labeled mitoses; SM, Smith-Martin; CI, confidence interval.

may be hard to distinguish between the parameters estimated from different models (see Section 5.2). Second, the data may be insufficient to unambiguously determine parameters of the model, that is, several parameter combinations may fit the data equally well.

To formulate a biologically reasonable specific model of cell division, one must qualitatively describe the mechanisms that govern cell division and cell death. For example, a very elegant quantitative model of cell turnover was formulated by Smith and Martin (Smith and Martin, 1973) in their quantitative study of the FLM (fraction of labeled mitoses) curves in cell culture. In the Smith-Martin (or simply SM) model, the progression of cells through the cell cycle involves a stochastic recruitment of cells from an A state (approximately corresponding to G1 phase of the cell cycle) into the dividing B phase (approximately equivalent to the S, G2, and M phases of the cycle). The dividing phase has a fixed duration Δ. The recruitment of cells from the A-state into the B-phase occurs at the fixed rate λ (the waiting time in the A state has exponential distribution with the parameter λ). The two parameters λ and Δ provide a complete description of cell division (Cain and Chao, 1997a,b). While the SM model could be viewed as a reasonable first approximation for the process of cell division, alternative models consistent with the FLM data have also been proposed (Castor, 1980; Brooks et al., 1980; Grasman, 1990).

In contrast with cell division, our understanding of processes that regulate cell death during the cell cycle is much poorer[2]. The simplest reasonable model might be with cell death occuring at constant rates d_A and d_B during A-state and B-phase, respectively. alternatively, cell death events may be restricted to discrete checkpoints within the A-state and B-phase. Therefore, it is crucial to understand the consequences of making an incorrect assumption for the death process.

In Section 5.2, we discuss some of the limitations of the widely used random birth-death model for the analysis of BrdU and CFSE data (Mohri et al., 1998; Bonhoeffer

[2]The problem of understanding cell death in comparison with cell division is long standing (Monod, 1949) and stems in part from the fact that cells which have undergone division can be visualized, while dead cells rapidly disappear being eliminated by apoptosis.

et al., 2000; Mohri et al., 2001; Revy et al., 2001). We illustrate how different assumptions on the timing of death in the cell cycle (as described above) can drastically alter our estimates for the parameters describing cell division and cell death. One potential way to solve this problem might be to estimate the parameters of the SM model by fitting the sm model to the CFSE data.

This approach has two limitations.

1. It assumes that the SM model is correct. As we discussed above, while the progression through the cell division cycle is biologically reasonable we do not know if the assumption that the death rates are constant during the A-state and B-phase is correct.

2. The data on the total cell numbers and CFSE distributions is insufficient to estimate all four parameters (λ, Δ, and death rates d_A, d_B).

In this paper, we use a different approach to solving the problem of estimation of parameters describing cell division and death from the CFSE data. We propose a general model of the cell cycle which makes no specific assumptions about the timing of cell division and death except to say that the probability of division and death are functions of the time since the previous division. We develop a method of rescaling (Section 5.3) to analyze this general model and show how this method allows us to identify and estimate kinetic parameters of the cell cycle that are *independent* of the specific mechanisms of cell division and cell death. In Section 5.4 we explain, with an example, how our method can be used. We will make available online a simple Mathematica program implementing our method for other datasets. Finally, we go over the limitations of the current techniques in the discussion section.

5.2 Illustrating the problem

To illustrate the consequences of making different specific assumptions about the death process, we consider two very simple models of the cell cycle. Both models are derived from the SM model with a very short B-phase (for derivations see Appendix). In both models, we let λ equal the rate of recruitment of cells from the A-state, and the average division time (i.e. the mean duration of the cell cycle) is given by $T = \frac{1}{\lambda}$. In the first model, we let d equal the death rate in the A-state and assume that there is no death in the B-phase. This model serves as an approximation to the widely used random birth-death model (Veiga-Fernandes et al., 2000; Bonhoeffer et al., 2000; Revy et al., 2001; Mohri et al., 2001) where the probability rates of both cell division and death are constant. The equations for $x_n(t)$, the number of cells in the n^{th} division at time t are described by

$$\frac{dx_n(t)}{dt} = 2\lambda x_{n-1}(t) - (\lambda + d)x_n(t),\ x_0(0) = x_0. \tag{5.1}$$

The mathematical solution to (5.1) can be written as

$$x_n(t) = \underbrace{\frac{(2\lambda t)^n}{n!}e^{-2\lambda t}}_{\text{distribution}}\underbrace{\left[x_0 e^{(\lambda - d)t}\right]}_{\text{total \#}}. \tag{5.2}$$

The first model therefore predicts that the the total number of cells grows exponentially at the rate $(\lambda - d)$. The distribution in the number of cells that have undergone n divisions by time t is Poisson, with the mean number of divisions that increases at the rate 2λ over time, independently of the death rate. In the second model, we let λ equal the rate of recruitment of cells from the A-state, but allow death to occur only in the very short B-phase, resulting in the mortality of a fraction f of cells in the B-phase. This model can be described by

$$\frac{dx_n(t)}{dt} = 2\lambda(1 - f)x_{n-1}(t) - \lambda x_n(t),\ x_0(0) = x_0. \tag{5.3}$$

The mathematical solution to (5.3) is

$$x_n(t) = \underbrace{\frac{(2\lambda(1-f)t)^n}{n!}e^{-2\lambda(1-f)t}}_{\text{distribution}}\underbrace{\left[x_0 e^{\lambda(1-2f)t}\right]}_{\text{total \#}} \tag{5.4}$$

Thus in the second model, the total number of cells grows exponentially at the rate $\lambda(1-2f)$. The distribution in the number of cells that have undergone n divisions by time t is again Poisson, but the mean number of divisions now increases at the rate $2\lambda(1-f)$ over time.

We see that in both models the distribution in the number of cells that have undergone n divisions is Poisson. In both models, the mean generation time is given by $T = 1/\lambda$. In the first model, the rate of increase in the mean number of divisions depends on λ whereas in the second model it depends on both λ and f. Consequently, if we were to estimate the mean generation time T using (5.1) or (5.3), we would obtain different estimates depending on the underlying model. For example, in case of a stable population ($\lambda = d$ in (5.1) and $f = 0.5$ in (5.3)) we would obtain a two fold discrepancy in the estimate for T. Such discrepancies will be even more pronounced for contracting populations. On the other hand, the estimates obtained from both models would be equally "significant" because both models produce equally good fits to the data.

This example illustrates that in the absence of further knowledge on the nature of the death process, we cannot estimate T with much confidence using only the data on the total number of cells and the number of divisions they have undergone (such as given by the CFSE data, for example). Given the uncertainties we have in the formulation of a specific model, we would like to define quantities that describe the cell turnover independently of the underlying model. In the following section, we do this by formulating a very general model of the cell cycle and determining which quantities we can estimate from the CFSE data.

5.3 General stage-structured model of cell cycle

5.3.1 Formulation

We postulate a set of relatively general biological assumptions about the nature of cell proliferation. We define cell *division* as an event when one mother cell leaves its generation and at the same time two identical daughter cells enter the next generation[3]. We define cell *death* as an event when one cell leaves its generation and no daugther cells are produced. Using this terminology, we assume that (i) The cycle of a given cell terminates either by cell division or by cell death. (ii) Cell division and cell death are independent random events whose probabilities of occurence depend only on the time since the cell was born. Specifically, such probabilities are independent of a particular cell and a given generation. (iii) The probability that cell division and cell death occur simultaneously is negligibly small. (iv) The system is closed, that is, new cells enter the population only through division and cells leave the circulating pool only through death.

We introduce the nomenclature for the stage-structured model. We let $x_n(t,s)$ denote the density of cells at time t that entered the n-th generation at time $t-s$. We refer to s as the age of cells inside the generation. We let $\lambda(s)$ denote the probability rate of cell division at age s and $d(s)$ denote the probability rate of cell death at age s inside the generation. The dynamics of the cell density within the n-th generation is described by the partial differential equation

$$\frac{\partial x_n(t,s)}{\partial t} + \frac{\partial x_n(t,s)}{\partial s} = -(\lambda(s)+d(s))x_n(t,s),\ n \geq 0. \tag{5.5}$$

The number of cells in the n-th generation that divide anywhere between the times t and $t+dt$ is given by

$$\left(\int_0^\infty \lambda(s)x_n(t,s)\ ds\right) dt,$$

[3]We refer to the cells which have undergone n divisions as cells in the n^{th} generation.

and therefore twice the number of cells enter the $n+1$-st generation between t and $t+dt$. Consequently, the dynamics of two consecutive generations are coupled through the boundary condition

$$x_n(t,0) = 2\int_0^\infty \lambda(s)x_{n-1}(t,s)\ ds,\ n \geq 1. \tag{5.6}$$

The set of equations (5.5-5.6) constitutes the general stage-structured model of the cell cycle. Since the system is closed, no cells enter the 0-th generation, that is, $x_0(t,0) = 0$.

The dynamics of the cell density is governed by a set of linear partial differential equations where the boundary condition (5.6) describes the rate at which cells enter the n-th generation. Therefore, rescaling this rate by a factor of $a \geq 0$ will result in the identical rescaling of the cell density $x_n(t,s)$. Equivalently, the dynamics of the rescaled cell densities $x_n(t,s,a) = a^n x_n(t,s)$ must satisfy the equations

$$\frac{\partial x_n(t,s,a)}{\partial t} + \frac{\partial x_n(t,s,a)}{\partial s} = -(\lambda(s)+d(s))x_n(t,s,a). \tag{5.7}$$

$$x_n(t,0,a) = 2a\int_0^\infty \lambda(s)x_{n-1}(t,s,a)\ ds. \tag{5.8}$$

The set of equations (5.7-5.8) constitutes the *rescaled* model of the cell cycle.

The biological meaning of the rescaling is intuitively simple: suppose that each mother cell produces not 2 but $2a$ daughter cells, then the cell density in each subsequent generation will be rescaled by a factor of a relative to the cell density in the preceding generation. In particular, the cell density in th n-th generation will be rescaled by a factor of a^n. Importantly, such rescaling does not affect the distribution of division or death events within a given generation, but only the rate of transfer of cells from one generation to the next.

In the next section, we show that for any nonnegative value of a, the rescaled model (5.7-5.8) describes an exponentially growing or decaying population. We also show how one can estimate parameters describing cell division and death from the relationship between the net proliferation rate $r(a)$ of the rescaled population and the value of a.

5.3.2 Method of rescaling

A typical set of experimental data (e.g., obtained from CFSE experiments) is presented as a time series for each generation of cells. We let $X_n(t) = \int_0^\infty x_n(t,s)ds$ denote the total number of cells in n-th generation at time t and consider the rescaled time series $X_n(t,a) = a^n X_n(t)$ with $a \geq 0$. In the previous section, we explained that the time series $X_n(t)$ corresponds to the original model (5.5-5.6) if and only if the rescaled time series $X_n(t,a)$ corresponds to the rescaled model (5.7-5.8). The total population sizes are given by

$$X(t) = \sum_{n=0}^{\infty} X_n(t), \quad X(t,a) = \sum_{n=0}^{\infty} a^n X_n(t),$$

respectively. Since (5.5-5.6) is a special case of (5.7-5.8) with $a = 1$, we analyze the more general case $a \geq 0$.

According to equations (5.7-5.8), the dynamics of the rescaled total cell density $x(t,s,a) = \sum_{n=0}^{\infty} a^n x_n(t,s)$ satisfies the classical von Foerster equation (von Foerster, 1959),

$$\frac{\partial x(t,s,a)}{\partial t} + \frac{\partial x(t,s,a)}{\partial s} = -(\lambda(s) + d(s))x(t,s,a), \tag{5.9}$$

$$x(t,0,a) = 2a \int_0^\infty \lambda(s)x(t,s,a)\ ds. \tag{5.10}$$

This model is also equivalent to the celebrated Lotka renewal equation (Sharpe and Lotka, 1911) widely used in demographical applications (Keyfitz, 1985b,a). The model (5.9-5.10) is linear, and therefore it predicts an asymptotically exponential net proliferation of $X(t,a)$ at a rate $r(a)$ which will vary as we vary the value a (Bellman and Cooke, 1963). The underlying relationship between $r(a)$ and a is given by the characteristic equation of the rescaled model

$$1 = 2a \int_0^\infty \lambda(s)e^{-\Lambda(s)-D(s)}e^{-r(a)s}ds, \tag{5.11}$$

where $\Lambda(s) = \int_0^s \lambda(z)\ dz$ and $D(s) = \int_0^s d(z)\ dz$ (Sharpe and Lotka, 1911) (We provide the derivation of (5.11) in the Appendix). Note that $r(a)$ is a strictly increasing function of a for $a \geq 0$.

In demographic applications, the functions $\Lambda(s)$ and $D(s)$ can be readily obtained from the census data (Keyfitz, 1985a) which provides the age distrbution of individuals

within one generation. In our application, the absence of such information (i.e. the age distribution of cells within a single generation) is a major obstacle. Fortunately, the CFSE data provide the age distribution of cells according to their division numbers. The novelty and the advantage of the rescaling method is that it allows one to approximate the characteristic equation (5.11) only using the distribution of cells by division numbers. To approximate the characteristic equation from a given experimental time series $X_n(t)$, we generate a family of rescaled time series $X_n(t,a)$, calculate the change in the total population size $X(t,a)$ with time, and evaluate the exponential proliferation rate $r(a)$ for each value of a. As a result, we can obtain the function $r(a)$ by manipulating a single time series of CFSE data.

5.3.3 Estimation of intrinsic parameters

With no additional assumptions on the model (5.5-5.6), we can estimate several intrinsic kinetic parameters of the cell cycle. Estimation of intrinsic parameters can be performed without specific knowledge of functions $\lambda(s)$ and $d(s)$. Such parameters include:

1. the fraction of cells that die in one generation (δ);

2. the mean generation time of surviving cells (τ).

Suppose that a cohort of N_0 cells enters a given generation (simultaneously) at $t = 0$. Equation (5.9) implies that the fraction of cells that have not divided and remain alive by time s is equal to $N(s) = N(0)\exp(-\Lambda(s) - D(s))$. Consequently, the number of cells in this cohort that will eventually divide is given by

$$N_1 = \int_0^\infty \lambda(s)N(s)\ ds = N_0 \int_0^\infty \lambda(s)e^{-\Lambda(s)-D(s)}ds.$$

Assuming that in the absence of death all cells eventually divide (that is, $\int_0^\infty \lambda(s)e^{-\Lambda(s)}\ ds = 1$), we find that the fraction of cells that die in one generation is given by $\delta = 1 - \frac{N_1}{N_0}$. Consequently,

$$\delta = 1 - \int_0^\infty \lambda(s) e^{-\Lambda(s)-D(s)} ds. \tag{5.12}$$

Similarly, the fraction of cells that survive through one generation (and therefore divide) is given by

$$1 - \delta = \int_0^\infty \lambda(s) e^{-\Lambda(s)-D(s)} ds.$$

Let a^* be the real root of r, that is, $r(a^*) = 0$. Equations (5.11) and (5.12) imply that

$$\delta = 1 - \frac{1}{2a^*}, \quad 1 - \delta = \frac{1}{2a^*}. \tag{5.13}$$

If not all cells die during one generation (i.e., $\delta < 1$), we can define the mean generation time for surviving cells as

$$\tau = \frac{1}{1-\delta} \int_0^\infty s\lambda(s) e^{-\Lambda(s)-D(s)} ds. \tag{5.14}$$

In the Appendix, we show that

$$\tau = \frac{1}{a^* r'(a^*)}. \tag{5.15}$$

According to (5.13) and (5.15), the quantities δ and τ can be estimated from a given experimental time series by first finding a^* as the a-intercept of the graph $r = r(a)$ and then finding the slope to this graph at $a = a^*$.

In addition to the mean generation time for surviving cells τ, we can estimate the variance of τ, that is, the variance of generation times for surviving cells. Such variance is mathematically expressed as

$$\sigma_\tau^2 = \frac{1}{1-\delta} \int_0^\infty s^2 \lambda(s) e^{-\Lambda(s)-D(s)} ds - \tau^2.$$

In the Appendix, we show that σ_τ^2 is given by

$$\sigma_\tau^2 = \tau^2 \left(1 + (a^*)^2 r''(a^*)\tau\right). \tag{5.16}$$

5.4 Illustration of the rescaling method

In this section, we analyze the time series for the dynamics of CFSE labeled P-14 transgenic naive CD8 T cells after adoptive transfer into irradiated hosts (Figure 1 in (Murali-Krishna and Ahmed, 2000)). The original data show that for the first week after transfer, cells grow exponentially ($r \approx 0.28\ day^{-1}$) and the mean number of divisions they have undergone increases approximately linearly. The CFSE data is best represented as the table of the number of cells having undergone n divisions at time t (Table 1).

		t (days)			
	$x_n(t)$	**0.5**	**1.25**	**3**	**8**
n (divisions)	**0**	7.38	7.07	1.77	0.0
	1	0.0	0.64	6.10	0.29
	2	0.0	0.0	6.58	5.71
	3	0.0	0.0	1.28	19.97
	4	0.0	0.0	0.0	18.83
	5	0.0	0.0	0.0	7.99
	6+	0.0	0.0	0.0	4.00

Table 5.1: The dynamics of P-14 Tg CD8 T cells after adoptive transfer into irradiated hosts (Murali-Krishna and Ahmed, 2000). The numbers above equal the number of cells per spleen divided by 10^4.

To estimate the model independent parameters δ and τ we need to rescale the data by a, where a varies from 0 upwards. This is done as follows: we multiply the number of cells in division class n by a^n, for example for $a = 2$ the number of cells in each division class is multiplied by 2^n; in Table 5.1 for day 3 the rescaled numbers will be $(1.77\,10^4)\cdot 2^0$, $(6.10\,10^4)\cdot 2^1$, $(6.58\,10^4)\cdot 2^2$, $(1.28\,10^4)\cdot 2^3$ and $0.0\cdot 2^4$. Following recalculation of the entire Table 5.1 for each a, and summing the number of cells in each division, the exponential growth rate $r(a)$ can be computed for that value of a (see Figure 5.1). From the plot of

$r(a)$ as a function of a we estimate a^* which equals the a-intersept and $r'(a^*)$ which equals the slope of $r(a)$ at a^*, allowing us to estimate δ and τ using equations (5.13) and (5.15) respectively.

We applied the rescaling method to the data on the dynamics of Tg CD8 T cells during proliferation in lymphopenic hosts to estimate the relationship between a and $r(a)$. For a list of values a (from $a = 0.2$ to $a = 1$), we fitted the logarithmic time series $\ln(X(t, a))$ with a linear function and used the resulting slope as the estimate for $r(a)$ (Figure 5.1). The estimated graph of a vs. $r(a)$ is shown by a bold solid line in Figure 5.2. The thin dashed lines represent the 67% confidence intervals for $r(a)$ which were calculated as standard errors for estimated rate r at a given a.

The value a^* at which the rate of exponential increase is zero is 0.549. Thus we estimated $a^* = 0.549$ with 67% confidence intervals $(0.546, 0.551)$ (these values are a-intercepts of 67% CI for $r(a)$, i.e., values at which dashed curves in Figure 5.2 intercept zero). Consequently, we can estimate $\delta = 0.089$ with the same small error ($CI = (0.085, 0.092)$). Thus, approximately 9% of the cells die on average during a single cell cycle in these experimental settings.

We estimated the slope of the function $r(a)$ at $a^* = 0.549$ using a five-point difference scheme (from $a^* - 2\Delta a$ to $a^* + 2\Delta a$ with a step $\Delta a = 0.02$; other small steps gave approximately the same result) and found that $r'(a^*) = 0.782$. To obtain the confidence intervals for $r'(a^*)$, we again used the five-point difference scheme but now for 67% CI of $r(a)$ to calculate the slopes $r'_+(a^*)$ and $r'_-(a^*)$. We found the 67% confidence intervals for $r'(a^*)$ to be: $(0.733, 0.831)$. Next, we estimated the the mean generation time $\tau = \frac{1}{a^* r'(a^*)}$ as $\tau = 2.33$ days. The 67% confidence intervals for τ were $(2.18, 2.50)$. From this CFSE time series we can conclude that following transfer of CD8 T cells into irradiated hosts approximately 9% die per cell cycle, and the average division time for surviving cells is approximately 2.3 days.

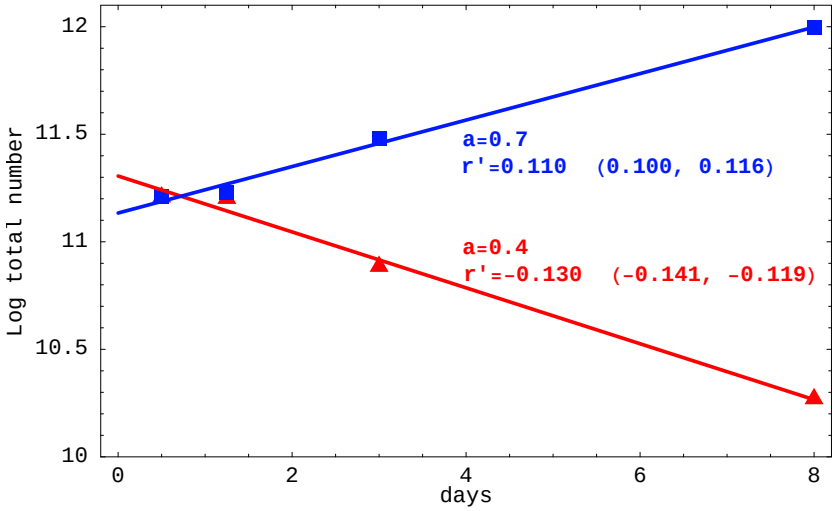

Figure 5.1: The change in the total population size of the rescaled population (with $a = 0.4$ and $a = 0.7$) with time using the data in Table 5.1. The regression lines for the total size increase are shown (r' is the mean slope; the 67% confidence intervals for the slope are in brackets).

5.5 Discussion

We have shown how the existing experimental data on the dynamics of cell populations and the number of divisions they have undergone can give us a quantitative picture of the underlying processes of cell division and cell death. We have argued that the problem is harder than previously thought (Gett and Hodgkin, 2000; Veiga-Fernandes et al., 2000; Revy et al., 2001). The reason is that making accurate estimates of parameters requires having a biologically reasonable quantitative model of both cell division and cell death. While the features of cell division can be quantitatively described (at least to a first approximation) much less is known about cell death. We have shown that choosing different, biologically plausible scenarios for cell death can lead to large inaccuracies in the estimation of the parameters of **both** cell division and cell death.

As the next step, we have investigated a general class of stage structured models, where the division and death of a cell are random variables that depend only on the time since cell division. For this class of models, we have introduced the method of rescaling, identified

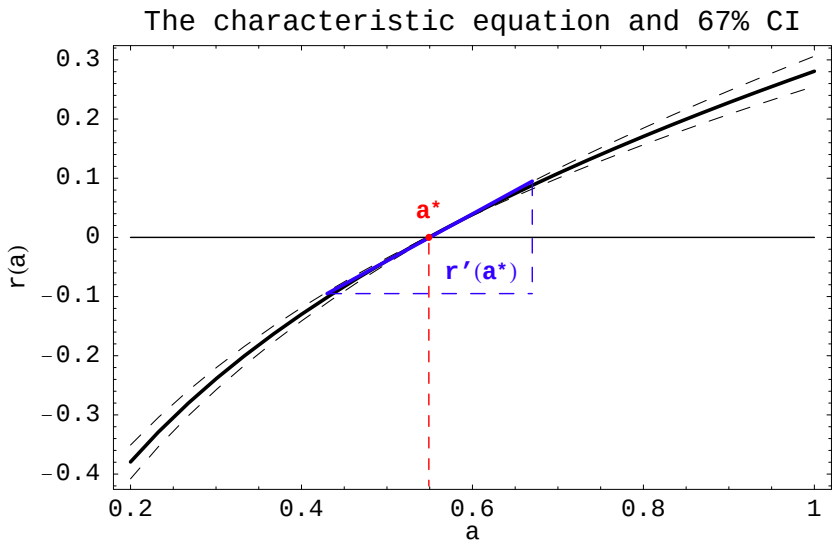

Figure 5.2: The plot of a vs. $r(a)$ obtained from the Tg cells data (see Table 5.1). The bold solid line is the graph $r = r(a)$ obtained from the data. The 67% confidence intervals for $r(a)$ are represented by the thin dashed lines. From this graph, we estimated the average survival as $1 - \delta = 0.91$ and the mean generation time of surviving cells $\tau = 2.33$ days.

parameters that are independent of the particular mechanisms of cell division and cell death, and shown how they can be estimated from the CFSE data. These parameters are the fraction of cells which die per cell cycle (δ) and the average time that surviving cells take to complete the cell cycle (τ). We should, however, note that these are not the only invariant parameters, but have been chosen because they have a clear biological interpretation. Other parameters such as the variance of generation times for surviving cells σ_τ^2 can also be obtained from the characteristic equation $r = r(a)$.

Due to the linearity of the model (5.9-5.10), the total population size $X(t)$ should acquire a phase of exponential change after a short transient (Bellman and Cooke, 1963). The rescaling method is based on the assumption that the population is in an exponential phase of its growth, and therefore it can be applied only to the data in which the total population size changes exponentially (i.e., increases, declines, or stays constant). Using the CFSE data we can calculate the rate of exponential increase of the total population size

$r(a)$ if each cell divides not in 2 but in $2a$ daughter cells for each value of a by renormalizing the CFSE data. The value of a at which the rescaled population size does not change (a^*), the slope and curvature of the experimental curve $r(a)$ at this point ($r'(a^*)$ and $r''(a^*)$) allow us to estimate δ, τ, and σ_τ^2.

The rescaling method allows us to estimate the invariant parameters in a very general case where we make no specific assumptions about how and when cells divide and die. Once a more detailed mechanistic model of cell division and death is validated then the parameters of such a model could be estimated. However, our present lack of information about cell death precludes formulating such a model.

Limitations of the rescaling method

The rescaling method is considerably more general than existing methods to analyze cell turnover. Nevertheless, it has several important limitations.

1. The CFSE assay can only detect up to a maximum of 5-10 divisions (Lyons, 2000) (after this, the CFSE dye becomes so dilute that it cannot be detected above the background) and this causes truncation errors. The truncation errors result from excluding cell counts with higher division numbers from the total population. It is therefore important that the CFSE measurements are obtained prior to such truncations having occurred.

2. In formulating the general model of cell turnover and the rescaling method we assume that the cell turnover is independent of both time and number of divisions. This may not be always valid, for example, as in the case of programmed immune responses (Kaech and Ahmed, 2001; van Stipdonk et al., 2001).

3. The rescaling method evaluates the function $r(a)$ assuming that the total population size changes exponentially and disregards the transient effects.

4. The rescaling method cannot be used to analyze the data obtained with such commonly used labels of cell division as BrdU or *D*-glucose because these labels do not

allow the quantification of the number of divisions that cells have undergone. Thus it is easier to point out the limitations of the earlier models for the analysis of BrdU data than to suggest ways in which the analysis can be improved.

5. The rescaling method described can only be applied to a homogenous population of cells. Some of the problems associated with measurement of turnover of heterogenous populations of cells using BrdU labeling have been elegantly considered by Asquith and coworkers (Asquith et al., 2002).

Existing analysis of CFSE data

Prior to this work, the CFSE data has been interpreted using two different approaches. For completeness, we describe them here. One approach is based on a purely statistical description of the data. Such description includes the dynamics of the mean number of divisions for surviving cells, the variance in the number of divisions for surviving cells, and the changes in the total number of precursors (obtained by dividing the number of cells undergone n divisions by 2^n) (Gett and Hodgkin, 2000). The model of cell division and death used by Gett and Hodgkin (2000) may serve as an alternative to the model presented in this paper. Gett and Hodgkin analyzed the CFSE data assuming that once a cell commits to division, it continues to divide in equal intervals of time and concluded that the rate at which cells commit to division follows a normal curve. These two models can be distinguished by qualifying the variance in the number of divisions for surviving cells. The general model of the cell cycle predicts a permanent increase in the variance while the model of Gett and Hodgkin predicts that after a transient increase this variance will remain nearly constant. Another approach is to fit the random birth-death model to the CFSE data (Veiga-Fernandes et al., 2000; Revy et al., 2001). This approach includes the estimation of the division and death rates (assuming that they are constant). We have demonstrated that the parameter estimation performed in this fashion largely depends on the specific underlying model of cell division and death. Discrepancies in the underlying model often lead to large inaccuracies in the resulting estimates. In particular, we note

that broad inferences such as that of Revy *et. al.* (2001) suggesting that "relative sizes of the CFSE peaks do not depend on the death rate and can be used to unambiguously determine the mean division time", are not generally valid.

We have emphasized our lack of a quantitative understanding of cell death and explained why the CFSE data alone cannot provide any insights into the regulation of cell death during the cell cycle. We hope that this theory will be instrumental in designing direct experimental studies that would elucidate such mechanisms of regulation. For instance, *in vitro* FLM studies of immune cell populations in expansion and contraction phases might give us a better understanding of where cell death occurs in the cell cycle. Such studies might allow us to generate a biologically valid explicit model for cell death and stimulate the development of quantitative methods for the estimation of the specific parameters of the model from the data.

5.6 Appendix: Mathematical derivations

5.6.1 Derivation of two limiting cases of the SM model

The original SM model is formulated as a set of differential equations:

$$\frac{dA_n(t)}{dt} = 2b_{n-1}(t,\Delta) - (\lambda + d_A)A_n(t), \tag{5.17}$$

$$\frac{\partial b_n(t,s)}{\partial t} + \frac{\partial b_n(t,s)}{\partial s} = -d_B b_n(t,s),\ 0 < s < \Delta,\ b_n(t,0) = \lambda A_n(t), \tag{5.18}$$

where n is the number of divisions that a given cell has undergone by time t. $A_n(t)$ is the number of cells in the A-state and $b_n(t,s)$ is the fraction of cells that entered the B-phase at time $t-s$. The amount of time that cells spend in the A-state is exponentially distributed with parameter λ. All cells spend a constant amount of time (Δ) in the B-phase. The mean generation time (i.e. the mean duration of the cell cycle) is given by $T = \frac{1}{\lambda} + \Delta$. All cells divide when they reach the end of the B-state and their daughter cells immediately re-enter the A-state of the next generation. The total number of cells in the B-phase is

given by $B_n(t) = \int_0^\Delta b_n(t,s)\ ds$. The death rates in A-state and B-phase are constant and denoted by d_A and d_B. One can reduce the SM model by expressing $b_n(t,s)$ in terms of $A_n(t)$ as

$$b_n(t,s) = \lambda e^{-d_B s} A_n(t-s),\ 0 < s < \Delta, \tag{5.19}$$

and replace equations (5.17-5.18) by

$$\frac{dA_n(t)}{dt} = 2\lambda e^{-d_B \Delta} A_{n-1}(t-\Delta) - (\lambda + d_A)A_n(t).. \tag{5.20}$$

$$B_n(t) = \lambda \int_0^\Delta e^{-d_B s} A_n(t-s)\ ds. \tag{5.21}$$

In (5.20), the factor $e^{-d_B \Delta}$ is the fraction of cells that survive in the B-phase.

Now we consider two limiting cases of the model (5.20-5.21) as Δ becomes infinitesimally small. Letting $x_n(t) = A_n(t) + B_n(t)$, we observe that due to equation (5.21), in the limiting case $\Delta \to 0$ we also have $A_n(t) \to x_n(t)$ and $A_{n-1}(t-\Delta) \to x_{n-1}(t)$.

Model 1. In the first limiting case (Model 1), we let $d = d_A = d_B$ so that the death rate is constant throughout the cell cycle. In the limit $\Delta \to 0$, equation (5.20) is replaced by

$$\frac{dx_n(t)}{dt} = 2\lambda x_{n-1}(t) - (\lambda + d)x_n(t),\ x_0(0) = x_0. \tag{5.22}$$

Model 2. In the second limiting case (Model 2), we let $d_A = 0$ so that there is no cell mortality in the A-state and assume that $e^{-d_B \Delta} \to 1 - f$ as $\Delta \to 0$. Here f is a limiting fraction of cells that die in the B-phase. This model is described by

$$\frac{dx_n(t)}{dt} = 2\lambda(1-f)x_{n-1}(t) - \lambda x_n(t),\ x_0(0) = x_0. \tag{5.23}$$

5.6.2 The characteristic equation

To derive the characteristic equation of (5.9-5.10), we substitute $x(t,s,a) = y(s,a)e^{r(a)t}$ into (5.9). Since the partial derivatives of $x(t,s,a)$ are given by

$$\frac{\partial x(t,s,a)}{\partial t} = r(a)y(s,a)e^{r(a)t}, \quad \frac{\partial x(t,s,a)}{\partial s} = \frac{\partial y(s,a)}{\partial s}e^{r(a)t},$$

$y(s,a)$ is a solution of

$$\frac{\partial y(s,a)}{\partial s} = -(\lambda(s) + d(s) + r(a))y(s,a), \ s \geq 0.$$

Solving this equation for y, we find that

$$y(s,a) = y(0,a)e^{-\Lambda(s)-D(s)}e^{-r(a)s}, \tag{5.24}$$

where $\Lambda(s) = \int_0^s \lambda(z)\,dz$ and $D(s) = \int_0^s d(z)\,dz$. Substituting (5.24) into (5.10), we obtain the equation

$$e^{r(a)t}y(0,a) = 2ae^{r(a)t}y(0,a)\int_0^\infty \lambda(s)e^{-\Lambda(s)-D(s)}e^{-r(a)s}\,ds.$$

Since $e^{r(a)t}y(0,a) \neq 0$, we cancel this quantity on both sides of the above equation to obtain the final form of the characteristic equation

$$1 = 2a\int_0^\infty \lambda(s)e^{-\Lambda(s)-D(s)}e^{-r(a)s}\,ds. \tag{5.25}$$

5.6.3 The expressions for τ and σ_τ^2

To derive the expression for τ, we differentiate (5.25) with respect to a to obtain

$$0 = 2\int_0^\infty \lambda(s)e^{-\Lambda(s)-D(s)}e^{-r(a)s}\,ds - 2ar'(a)\int_0^\infty s\lambda(s)e^{-\Lambda(s)-D(s)}e^{-r(a)s}\,ds. \tag{5.26}$$

We substitute $a = a^*$ and $r(a^*) = 0$ into (5.26) and obtain

$$0 = \frac{1}{a^*} - 2a^*r'(a^*)(1-\delta)\tau.$$

Using the fact that $2a^*(1-\delta) = 1$, we find that

$$\tau = \frac{1}{a^*r'(a^*)}. \tag{5.27}$$

To derive the expression for σ_τ^2, we differentiate (5.26) with respect to a to obtain

$$\begin{aligned} 0 = &-2r'(a)\int_0^\infty s\lambda(s)e^{-\Lambda(s)-D(s)}e^{-r(a)s}\,ds \\ &+2a(r'(a))^2\int_0^\infty s^2\lambda(s)e^{-\Lambda(s)-D(s)}e^{-r(a)s}\,ds \\ &-2(r'(a)+ar''(a))\int_0^\infty s\lambda(s)e^{-\Lambda(s)-D(s)}e^{-r(a)s}\,ds. \end{aligned} \tag{5.28}$$

We substitute $a = a^*$, $r(a^*) = 0$, and (5.27) into (5.28) and find that

$$\frac{1}{1-\delta}\int_0^\infty s^2\lambda(s)e^{-\Lambda(s)-D(s)}ds = \tau^2(2 + (a^*)^2r''(a^*)\tau).$$

Finally, we compute

$$\sigma_\tau^2 = \frac{1}{1-\delta}\int_0^\infty s^2\lambda(s)e^{-\Lambda(s)-D(s)}ds - \tau^2 = \tau^2\Big(1 + (a^*)^2r''(a^*)\tau\Big). \tag{5.29}$$

5.7 Publication status

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Bibliography

Ancel Meyers, l., Levin, B. R., Richardson, A. R., and Stojilikovic, I. 2003. Epidemiology, hypermutation, within-host evolution and the virulence of *neisseria meningitidis*. *Proc Roy Soc Lond B* 270:1667–77.

Anderson, R. M. and May, R. M. 1982. Coevolution of hosts and parasites. *Parasitology* 85:411–426.

Anderson, R. M. and May, R. M. 1991. Infectious diseases in humans: dynamics and control. Oxford University Press, Oxford, UK.

Andre, J. B., Ferdy, J. B., and Godelle, B. 2003. Within-host parasite dynamics, emerging trade-off and evolution of virulence with immune system. *Evolution* 57:1489–1497.

Antia, R., Bergstrom, C. T., Pilyugin, S. S., Kaech, S. M., and Ahmed, R. 2003. Models of CD8+ responses: 1. What is the antigen-independent proliferation program. *J Theor Biol* 221:585–98.

Antia, R. and Koella, J. C. 1994. A model of non-specific immunity. *J Theor Biol* 168:141–50.

Antia, R., Levin, B. R., and May, R. M. 1994. Within-host population dynamics and the evolution and maintenance of microparsite virulence. *Am. Nat.* 144:457–472.

Antia, R. and Lipsitch, M. 1997. Mathematical models of parasite responses to host immune defenses. *Parasitology* 115:S155–67.

Antonovics, J. and Thrall, P. 1994. Cost of resistance and maintenance of genetic-polymorphism in host-pathogen systems. *Proceedings of the Royal Society, London, Series B* 257:105–110.

Arnaout, R. A., Lloyd, A. L., O'Brien, T. R., Goedert, J. J., Leonard, J. M., and Nowak, M. A. 1999. A simple relationship between viral load and survival time in HIV-1 infection. *Proc Natl Acad Sci U S A* 96:11549–53.

Arnaout, R. A., Nowak, M. A., and Wodarz, D. 2000. HIV-1 dynamics revisited: biphasic decay by cytotoxic T lymphocyte killing? *Proc R Soc Lond B Biol Sci* 267:1347–54.

Asquith, B., Debacq, C., Macallan, D., Willems, L., and Bangham, C. 2002. Lymphocyte kinetics: the interpretation of labelling data. *Trends Immunol* 23:596–601.

Bailey, N. T. J. 1975. The mathematical theory of infectious diseases and its application. Griffin, Inc., London, UK.

Ballabeni, P. 1995. Parasite-induced gigantism in a snail: a host adaptation? *Functional Ecology* 9:887–893.

Baudoin, M. 1975. Host castration as parasitic strategy. *Evolution* 29:335–352.

Behbehani, A. M. 1983. The smallpox story: life and death of an old disease. *Microbiol Rev* 47:455–509.

Bellman, R. and Cooke, K. L. 1963. Differential-difference equations. Academic Press.

Berche, P. 2001. The threat of smallpox and bioterrorism. *Trends Microbiol* 9:15–8.

Bergstrom, C. T., McElhany, P., and Real, L. A. 1999. Transmission bottlenecks as determinants of virulence in rapidly evolving pathogens. *Proc Natl Acad Sci U S A* 96:5095–100.

Best, S. M. and Kerr, P. J. 2000. Coevolution of host and virus: the pathogenesis of virulent and attenuated strains of myxoma virus in resistant and susceptible European rabbits. *Virology* 267:36–48.

Black, F. L. 1992. Why did they die? *Science* 258:1739–40.

Bonhoeffer, S., Lenski, R. E., and Ebert, D. 1996. The curse of the pharaoh: the evolution of virulence in pathogens with long living propagules. *Proc R Soc Lond B Biol Sci* 263:715–21.

Bonhoeffer, S., May, R., Shaw, G., and Nowak, M. 1997. Virus dynamics and drug therapy. *Proc Natl Acad Sci U S A* 94:6971–6976.

Bonhoeffer, S., Mohri, H., Ho, D., and Perelson, A. S. 2000. Quantification of cell turnover kinetics using 5-bromo-2'-deoxyuridine. *J Immunol* 164:5049–54.

Bonhoeffer, S. and Nowak, M. 1994a. Intra-host versus inter-host selection: Viral strategies of immune function impairment. *Proc Natl Aca Sci USA* 91:8062–8066.

Bonhoeffer, S. and Nowak, M. 1994b. Mutation and the evolution of virulence. *Proc. R. Soc. Lond. B.* 258:133–140.

Boots, M. and Bowers, R. 2003. Baseline criteria and the evolution of hosts and parasites: D0, R0 and competition for resources between strains. *J Theor Biol* 223:361–5.

Boots, M. and Sasaki, A. 1999. 'Small worlds' and the evolution of virulence: infection occurs locally and at a distance. *Proc R Soc Lond B Biol Sci* 266:1933–8.

Bowers, R. G. 1999. A baseline model for the apparent competition between many host strains: the evolution of host resistance to microparasites. *J Theor Biol* 200:65–75.

Bowers, R. G. 2001. The basic depression ratio of the host: the evolution of host resistance to microparasites. *Proc R Soc Lond B Biol Sci* 268:243–50.

Bremermann, H. J. and Thieme, H. R. 1989. A competetive exclusion principle for pathogen virulence. *J Math Biol* 27:179–190.

Brooks, R. F., Bennett, D. C., and Smith, J. A. 1980. Mammalian cell cycles need two random transitions. *Cell* 19:493–504.

Brown, S., Hochberg, M., and Grenfell, B. 2002. Does multiple infection select for raised virulence? *Trends Microbiol* 10:401–5.

Buchbinder, S., Katz, M., Hessol, N., O'Malley, P., and Holmberg, S. 1994. Long-term HIV-1 infection without immunologic progression. *AIDS* 8:1123–8.

Buckley, K. A., Li, P. L., Khimani, A. H., Hofmann-Lehmann, R., Liska, V., Anderson, D. C., McClure, H. M., and Ruprecht, R. M. 2003. Convergebt evolution of SIV *env* after independent inoculation of rhesus macaques with infectious proviral DNA. *Virology* 312:470–480.

Bucy, R. P. 1999. Immune clearance of HIV type 1 replication-active cells: a model of two patterns of steady state HIV infection. *AIDS Res Hum Retroviruses* 15:223–7.

Bucy, R. P., Hockett, R. D., Derdeyn, C. A., Saag, M. S., Squires, K., Sillers, M., Mitsuyasu, R. T., and Kilby, J. M. 1999. Initial increase in blood CD4(+) lymphocytes after HIV antiretroviral therapy reflects redistribution from lymphoid tissues. *J Clin Invest* 103:1391–8.

Bull, J., Molineux, I., and Rice, W. 1991. Selection of benevolence in a host - parasite system. *Evolution* 45:875–882.

Bull, J. J. 1994. Perspective - virulence. *Evolution* 48:1423–37.

Bull, J. J. and Molineux, I. J. 1992. Molecular genetics of adaptation in an experimenta model of cooperation. *Evolution* 46:882–95.

Burnet, M. and White, D. O. 1972. Natural history of infectious disease. Cambridge University Press, London, UK, forth edition.

Cain, S. J. and Chao, P. S. 1997a. Transition probability cell cycle model. 1. Balanced growth. *J theor biol* 185:55–67.

Cain, S. J. and Chao, P. S. 1997b. Transition probability cell cycle model. 2. Non-balanced growth. *J theor biol* 185:69–79.

Casazza, J. P., Betts, M. R., Picker, L. J., and Koup, R. A. 2001. Decay kinetics of human immunodeficiency virus-specific CD8+ T cells in peripheral blood after initiation of highly active antiretroviral therapy. *J Virol* 75:6508–16.

Castor, L. N. 1980. A G1 rate model accounts for cell-cycle kinetics attributed to 'transition probability'. *Nature* 287:857–9.

Chakrabarti, L. A., Lewin, S. R., Zhang, L., Gettie, A., Luckay, A., Martin, L. N., Skulsky, E., Ho, D. D., Cheng-Mayer, C., and Marx, P. A. 2000. Normal T-cell turnover in sooty mangabeys harboring active simian immunodeficiency virus infection. *J Virol* 74:1209–23.

Choo, K., Williams, P., and Day, T. 2003. Host mortality, predation and the evolution of parasite virulence. *Ecology letters* 6:310 – 315.

Chua, K., Bellini, W., Rota, P., Harcourt, B., Tamin, A., Lam, S., Ksiazek, T., Rollin, P., Zaki, S., Shieh, W., Goldsmith, C., Gubler, D., Roehrig, J., Eaton, B., Gould, A., Olson, J., Field, H., Daniels, P., Ling, A., Peters, C., Anderson, L., and Mahy, B. 2000. Nipah virus: a recently emergent deadly paramyxovirus. *Science* 288:1432–5.

Clayton, D. H. and Tompkins, D. M. 1994. Ectoparasite virulence is linked to mode of transmission. *Proc R Soc Lond B Biol Sci* 256:211–7.

Cocchi, F., DeVico, A. L., Garzino-Demo, A., Arya, S. K., Gallo, R. C., and Lusso, P. 1995. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV- suppressive factors produced by CD8+ T cells. *Science* 270:1811–5.

Cooper, V., Reiskind, M., Miller, J., Shelton, K., Walther, B., Elkinton, J., and Ewald, P. 2002. Timing of transmission and the evolution of virulence of an insect virus. *Proc R Soc Lond B Biol Sci* 269:1161–5.

Davies, C. M., Webster, J. P., and Woolhous, M. E. 2001. Trade-offs in the evolution of virulence in an indirectly transmitted macroparasite. *Proc R Soc Lond B Biol Sci* 268:251–7.

Davis, B. D., Dulbecco, R., Eisen, H. N., Ginsberg, H. S., and Wood, W. B. 1969. Microbiology. Harper & Row publisher, New York, NY.

Day, T. 2001. Parasite transmission modes and the evolution of virulence. *Evolution* 55:2389–400.

Day, T. 2002a. The evolution of virulence in vector-borne and directly transmitted parasites. *Theor Pop Biol* 62:199 – 213.

Day, T. 2002b. On the evolution of virulence and the relationship between various measures of mortality. *Proc R Soc Lond B Biol Sci* 269:1317–23.

Day, T. 2002c. Virulence evolution via host exploitation and toxin production in spore-producing pathogens. *Ecology letters* 5:471 – 476.

Day, T. 2003. Virulence evolution and the timing of disease life-history events. *Trends Ecol Evol* 18:113 – 118.

Day, T. and Burns, J. G. 2003. A consideration of patterns of virulence arising from host-parasite coevolution. *Evolution* 57:671–676.

De Leo, G. and Dobson, A. 2002. Virulence management in wildlife populations, pp. 413–424. *In* U. Dieckmann, J. A. J. Metz, M. W. Sabelis, and K. Sigmund (eds.), Adaptive dynamics of infectious disease: in pirsuit of virulence management. Oxford University Press.

Deacon, N., Tsykin, A., Solomon, A., Smith, K., Ludford-Menting, M., Hooker, D., McPhee, D., Greenway, A., Ellett, A., Chatfield, C., and et al. 1995. Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. *Science* 270:988–91.

Debacq, C., Asquith, B., Kerkhofs, P., Portetelle, D., Burny, A., Kettmann, R., and Willems, L. 2002. Increased cell proliferation, but not reduced cell death, induces lymphocytosis in bovine leukemia virus-infected sheep. *Proc Natl Acad Sci U S A* 99:10048–53.

Dieckmann, U. 2002. Adaptive dynamics of pathogen-host interactions, pp. 39–59. *In* U. Dieckmann, J. A. J. Metz, M. W. Sabelis, and K. Sigmund (eds.), Adaptive dynamics of infectious disease: in pirsuit of virulence management. Oxford University Press.

Dieckmann, U., Metz, J. A. J., Sabelis, M. W., and Sigmund, K. 2002. Adaptive dynamics of infectious disease: in pirsuit of virulence management. Oxford University Press.

Diop, O. M., Gueye, A., Dias-Tavares, M., Kornfeld, C., Faye, A., Ave, P., Huerre, M., Corbet, S., Barre-Sinoussi, F., and Muller-Trutwin, M. C. 2000. High levels of viral replication during primary simian immunodeficiency virus SIVagm infection are rapidly and strongly controlled in African green monkeys. *J Virol* 74:7538–47.

Dobson, C. and Owen, M. 1977. Influence of serial passage on the infectivity and immunogenicity of Nerospiroides dubius in mice. *Int J Parasit* 7:463–466.

Dubos, R. 1965. Man adapting. The Vail-Ballou Press, Binghamton, N.Y., USA.

Dwyer, G., Levin, S., and Buttel, L. 1990. A simulation model of the population dynamics and evolution of myxomatosis. *Ecological Monographs* 60:423–447.

Dyer, M. and Day, K. 2000. Commitment to gametocytogenesis in Plasmodium falciparum. *Parasitol Today* 16:102–7.

Ebert, D. 1994. Virulence and local adaptation of a horizontally transmitted parasite. *Science* 265:1084–86.

Ebert, D. 1995. The ecological interactions between a microsporidian parasite and its host Daphnia magna. *J Anim Ecol* 64:361–369.

Ebert, D. 1998. Experimental evolution of microparasites. *Science* 282:1432–5.

Ebert, D. 1999. The evolution and expression of parasite virulence, pp. 161–172. *In* S. C. Stearns (ed.), Evolution in health and disease. Oxford University Press, New York, NY.

Ebert, D. and Bull, J. 2003. Challenging the trade-off model for the evolution of virulence: is virulence management feasible? *Trends Microbiol* 11:15–20.

Ebert, D. and Hamilton, W. 1996. Sex against virulence: the coevolutin of parasitic diseases. *Trends Ecol Evol* 11:79–82.

Ebert, D. and Herre, E. 1996. The evolution of parasitic diseases. *Parasitology today* 12:96–101.

Ebert, D., Lipsitch, M., and Mangin, K. 2000. The effect of parasites on host population density and extinction: Experimental epidemiology with Daphnia and six microparasites. *Am Nat* 156:459–77.

Ebert, D. and Mangin, K. 1997. The influence of host demography on the evolution of virulence of a microsporidian gut parasite. *Evolution* 51:1828–37.

Ebert, D. and Weisser, W. W. 1997. Optimal killing for obligate killers: the evolution of life histories and virulence of semelparous parasites. *Proc R Soc Lond B Biol Sci* 264:985–91.

Elena, S. 2001. Evolutionary history conditions the timing of transmission in vesicular stomatitis virus. *Infect Genet Evol* 1:151–9.

Escrui, F., Fraile, A., and García-Arenal, F. 2003. Evolution of virulence of a plant virus. *Evolution* 57:755–65.

Evans, D. T., O'Connor, D. H., Jing, P., Dzuris, J. L., Sidney, J., da Silva, J., Allen, T. M., Horton, H., Venham, J. E., Rudersdorf, R. A., Vogel, T., Pauza, C. D., Bontrop, R. E., DeMars, R., Sette, A., Hughes, A. L., and Watkins, D. I. 1999. Virus-specific cytotoxic T-lymphocyte responses select for amino-acid variation in simian immunodeficiency virus Env and Nef. *Nat Med* 5:1270–6.

Ewald, P. 1983. Host-parasite relations, vectors, and the evolution of disease severity. *Annual Review of Ecology and Systematics* 14:465–485.

Ewald, P. 1987. Transmission modes and evolution of the parasitism-mutualism continuum. *Ann N Y Acad Sci* 503:295–306.

Ewald, P. 1988. Cultural vectors, virulence and the emergence of evolutionary epidemology. *Oxford Surveys in Evolutionary Biology* 5:215–245.

Ewald, P. 1991. Transmission modes and the evolution of virulence, with special reference to cholera, influenza and aids. *Human Nature* 2:1–30.

Ewald, P. 1993. The evolution of virulence. *Sci Amer* 268:56–62.

Ewald, P. 1994. Evolution of infectious disease. Oxford University Press, Oxford, NY, USA.

Ewald, P. W. and De Leo, G. 2002. Alternative transmission modes and the evolution of virulence, pp. 11–25. *In* U. Dieckmann, J. A. J. Metz, M. W. Sabelis, and K. Sigmund (eds.), Adaptive dynamics of infectious disease: in pirsuit of virulence management. Oxford University Press.

Felsenstein, J. 1988. Sex and evolution of recombination, pp. 74–86. *In* Richard E. Michod and Bruce R. Levin (ed.), The evolution of sex: an examination of current ideas, chapter 5. Sinauer Associates Inc., Sunderland, Massachusets.

Fenner, F. 1983. Biological control, as exemplified by smallpox eradication and myxomatosis. *Proceedings of the Royal Society, London, Series B* B 218:259–285.

Fenner, F., Day, M., and Woodroofe, G. 1956. The epidemiological consequences of the mechanical transmission of myxomatosis by mosquitoes. *J Hyg (Lond)* 54:284–303.

Fenner, F. and Fantini, B. 1999. Biological control of vertebrate pests: the history of myxomatosis; an experiment in evolution. CABI Publishing, Wallingford, oxon, uk; New York, NY, USA.

Fenner, F. and Ratcliffe, F. 1965. Myxomatosis. Cambridge university press, Cambridge, UK.

Ferguson, N. M., deWolf, F., Ghani, A. C., Fraser, C., Donnelly, C. A., Reiss, P., Lange, J. M., Danner, S. A., Garnett, G. P., Goudsmit, J., and Anderson, R. M. 1999. Antigen-driven CD4+ T cell and HIV-1 dynamics: residual viral replication under highly active antiretroviral therapy. *Proc Natl Acad Sci U S A* 96:15167–72.

Fine, P. 1975. Vectors and vertical transmission: an epidemiologic perspective. *Ann N Y Acad Sci* 266:173–94.

Finlay, B. B. and Falkow, S. 1997. Common themes in microbial pathogenicity revisited. *Microbiol Mol Biol Rev* 61:136–69.

Frank, S. A. 1992. A kin selection model for the evolution of virulence. *Proc R Soc Lond B* 250:195–197.

Frank, S. A. 1993. Recognition and polymorphism in host-parasite genetics. *Philos Trans R Soc Lond B Biol Sci* 346:283–93.

Frank, S. A. 1996. Models of parasite virulence. *Q. Rev. Biol.* 71:37–78.

Galvani, A. P. 2003. Epidemiology meets evolutionary ecology. *Trends Microbiol* 18:132–39.

Gandon, S. 1998. The curse of the pharaoh hypothesis. *Proc R Soc Lond B Biol Sci* 265:1545–52.

Gandon, S., Jansen, V. A., and van Baalen, M. 2001a. Host life history and the evolution of parasite virulence. *Evolution* 55:1056–62.

Gandon, S., Mackinnon, M., Nee, S., and Read, A. 2003. Imperfect vaccination: some epidemiological and evolutionary consequences. *Proc R Soc Lond B Biol Sci* 270:1129–36.

Gandon, S., Mackinnon, M. J., Nee, S., and Read, A. F. 2001b. Imperfect vaccines and the evolution of pathogen virulence. *Nature* 414:751–6.

Gandon, S. and Michalakis, Y. 2000. Evolution of parasite virulence against qualitative or quantitative host resistance. *Proc R Soc Lond B Biol Sci* 267:985–90.

Ganusov, V. 2003. Evolution of virulence: adaptive or not? *Trends Microbiol* 11:112–3.

Ganusov, V. V. and Antia, R. 2003. Trade-offs and evolution of virulence of microparasites: do details matter? *Theor Pop Biol* accepted to publication.

Ganusov, V. V., Bergstrom, C. T., and Antia, R. 2002. Within-host population dynamics and the evolution of microparasites in a heterogeneous host population. *Evolution* 52:213–23.

Gett, A. and Hodgkin, P. 2000. A cellular calculus for signal integration by T cells. *Nature Immunology* 1:239–244.

Gilchrist, M. and Sasaki, A. 2002. Modeling host-parasite coevolution: A nested approach based on mechanistic models. *J Theor Biol* 218:289–308.

Goto, Y., Nishimura, Y., Baba, K., Mizuno, T., Endo, Y., Masuda, K., Ohno, K., and Tsujimoto, H. 2002. Association of plasma viral RNA load with prognosis in cats naturally infected with feline immunodeficiency virus. *J Virol* 76:10079–83.

Grasman, J. 1990. A deterministic model of the cell cycle. *Bulletin of Mathematical Biology* 52:535–47.

Gray, R. H., Wawer, M. J., Brookmeyer, R., Sewankambo, N. K., Serwadda, D., Wabwire-Mangen, F., Lutalo, T., Li, X., vanCott, T., and Quinn, T. C. 2001. Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. *Lancet* 357:1149–53.

Grossman, Z., Polis, M., Feinberg, M. B., Levi, I., Jankelevich, S., Yarchoan, R., Boon, J., de Wolf, F., Lange, J. M., Goudsmit, J., Dimitrov, D. S., and Paul, W. E. 1999. Ongoing HIV dissemination during HAART. *Nat Med* 5:1099–104.

Guidotti, L. G. and Chisari, F. V. 2001. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* 19:65–91.

Gupta, S. and Hill, A. V. 1995. Dynamic interactions in malaria: host heterogeneity meets parasite polymorphism. *Proc R Soc Lond B Biol Sci* 261:271–7.

Haraguchi, Y. and Sasaki, A. 2000. The evolution of parasite virulence and transmission rate in a spatially structured population. *J Theor Biol* 203:85–96.

Hellerstein, M., Hanley, M. B., Cesar, D., Siler, S., Papageorgopoulos, C., Wieder, E., Schmidt, D., Hoh, R., Neese, R., Macallan, D., Deeks, S., and McCune, J. M. 1999. Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. *Nat Med* 5:83–9.

Herre, E. 1993. Population structure and the evolution of virulence in nematode parasites in Fig wasps. *Science* 259:1442–1445.

Herz, A. V., Bonhoeffer, S., Anderson, R. M., May, R. M., and Nowak, M. A. 1996. Viral dynamics in vivo: limitations on estimates of intracellular delay and virus decay. *Proc Natl Acad Sci U S A* 93:7247–51.

Hlavacek, W. S., Stilianakis, N. I., Notermans, D. W., Danner, S. A., and Perelson, A. S. 2000. Influence of follicular dendritic cells on decay of HIV during antiretroviral therapy. *Proc Natl Acad Sci U S A* 97:10966–71.

Ho, D., Neumann, A., Perelson, A., Chen, W., Leonard, J., and Markowitz, M. 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 373:123–126.

Imhoof, B. and Schmid-Hempel, P. 1998. Single-clone and mixed-clone infections versus host environment in Crithidia bombi infecting bumblebees. *Parasitology* 117 (Pt 4):331–6.

Jeon, K. 1972. Development of cellular dependence on infective organisms: micrurgical studies in amoebas. *Science* 176:1122–3.

Jin, X., Bauer, D. E., Tuttleton, S. E., Lewin, S., Gettie, A., Blanchard, J., Irwin, C. E., Safrit, J. T., Mittler, J., Weinberger, L., Kostrikis, L. G., Zhang, L., Perelson, A. S., and Ho, D. D. 1999. Dramatic rise in plasma viremia

after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J Exp Med* 189:991–8.

Kaech, S. M. and Ahmed, R. 2001. Memory CD8 T cells differentiation: initial antigen encounter triggers a developmental program in naive cells. *Nature Immunology* 2:415–421.

Kaech, S. M., Wherry, E. J., and Ahmed, R. 2002. Effector and memory T-cell differentiation: implications for vaccine development. *Nat Review Immunol* 2:251–262.

Kerr, P. and McFadden, G. 2002. Immune responses to myxoma virus. *Viral Immunol* 15:229–46.

Kerr, P., Merchant, J., Silvers, L., Hood, G., and Robinson, A. 2003. Monitoring the spread of myxoma virus in rabbit Oryctolagus cuniculus populations on the southern tablelands of New South Wales, Australia. II. Selection of a strain of virus for release. *Epidemiol Infect* 130:123–33.

Keyfitz, N. 1985a. Applied mathematical demography. Springer, New York.

Keyfitz, N. 1985b. Introduction to the mathematics of population. Addison-Wesley, Reading, MA.

Klenerman, P., Phillips, R. E., Rinaldo, C. R., Wahl, L. M., Ogg, G., May, R. M., McMichael, A. J., and Nowak, M. A. 1996. Cytotoxic T lymphocytes and viral turnover in HIV type 1 infection. *Proc Natl Acad Sci U S A* 93:15323–8.

Klenerman, P. and Zinkernagel, R. M. 1997. What can we learn about human immunodeficiency virus infection from a study of lymphocytic choriomeningitis virus? *Immunol Rev* 159:5–16.

Knell, R. J. 1999. Sexually transmitted disease and parasite-mediated sexual selection. *Evolution* 53:957–61.

Knolle, H. 1989. Host density and the evolution of parasite virulence. *J Theor Biol* 136:199–207.

Koenig, S., Conley, A. J., Brewah, Y. A., Jones, G. M., Leath, S., Boots, L. J., Davey, V., Pantaleo, G., Demarest, J. F., Carter, C., and et al. 1995. Transfer of HIV-1-specific cytotoxic T lymphocytes to an AIDS patient leads to selection for mutant HIV variants and subsequent disease progression. *Nat Med* 1:330–6.

Kruger, D., Ulrich, R., and Lundkvist, A. 2001. Hantavirus infections and their prevention. *Microbes Infect* 3:1129–44.

Lafferty, K. 1999. The evolution of trophic transmission. *Parasitol Today* 15:111–5.

Lednicky, J. A. 2003. Hantaviruse: a short review. *Arch Pathol Lab Med* 127:30–35.

Lenski, R. E. and May, R. M. 1994. The evolution of virulence in parasites and pathogens: reconciliation between two competing hypotheses. *J Theor Biol* 169:253–265.

Leung, B. and Forbes, M. R. 1998. The evolution of virulence: a stochastic simulation model examining parasitism at individual and population levels. *Evolutionary Ecology* 12:165–177.

Levin, B. 1996. The evolution and maintenance of virulence in microparasites. *Emerg Infec Dis* 2:93–102.

Levin, B. and Bull, J. 1994. Short-sighted evoluion and the virulence of pathogenic microorganisms. *Trends in Microbiology* 2:76–81.

Levin, B. and Svanborg-Eden, C. 1990. Selection and the evolution of virulence in bacteria: An eccumenical excursion and modest suggestion. *Parasitology* 100:S103–115.

Levin, B. R., Bull, J. J., and Stewart, F. M. 1996. The intrinsic rate of increase of HIV/AIDS: epidemiological and evolutionary implications. *Math Biosci* 132:69–96.

Levin, B. R., Bull, J. J., and Stewart, F. M. 2001. Epidemiology, evolution, and future of the HIV/AIDS pandemic. *Emerg Infect Dis* 7:505–11.

Levin, S. and Pimentel, D. 1981. Selection of intermediate rates of increase in parasite-host systems. *Am Nat* 117:308–315.

Levy, J. A. 1998. HIV and pathogenesis of AIDS. ASM Press, Washington, DC.

Lipsitch, M., Here, E., and Nowak, M. 1995a. Host population structure and the evolution of virulence: A "law of diminishing returns". *Evolution* 49:743–748.

Lipsitch, M. and Moxon, R. 1997. Virulence and transmissibility of pathogens: what is the relationship? *Trends Microbiol* 5:31–37.

Lipsitch, M., Nowak, M., Ebert, D., and May, R. 1995b. The population dynamics of vertically and horizontally transmitted parasites. *Proceedings of the Royal Society, London, Series B* 260:321–327.

Lipsitch, M., Siller, S., and Nowak, M. 1996. The evolution of virulence in pathogens with vertical and horizontal transmission. *Evolution* 50:1729–1741.

Lloyd, A. L. 2001. The dependence of viral parameter estimates on the assumed viral life cycle: limitations of studies of viral load data. *Proc R Soc Lond B Biol Sci* 268:847–54.

Longini, I. M. J. 1990. Modelling the decline of CD4+ T-lymphocyte coutns in HIV-infected individuals. *J AIDS* 3:930–931.

Longini, I. M. J., Clark, S. W., Byers, R. H., Ward, J. W., Darrow, W. W., Lemp, G. F., and Hethcote, H. W. 1989. Statistical analysis of the stages of HIV infection iusing a Markov model. *Stat Medicine* 8:831–43.

Lyons, A. and Parish, C. R. 1994. Determination of lymphocyte division by flow cytometry. *J Immunol Methods* 171:131–137.

Lyons, A. B. 2000. Analysing cell division in vivo and in vitro using flow cytometric measurement of cfse dye dilution. *J Immunol Methods* 243:147–54.

Mackinnon, M., Gaffney, D., and Read, A. 2002. Virulence in rodent malaria: host genotype by parasite genotype interactions. *Infect Genet Evol* 1:287–96.

Mackinnon, M. J. and Read, A. F. 1999. Genetic relationship between parasite virulence and transmission in the rodent malaria Plasmodium chabaudi. *Evolution* 53:689–703.

Magierowska, M., Theodorou, I., Debre, P., Sanson, F., Autran, B., Riviere, Y., Charron, D., and Costagliola, D. 1999. Combined genotypes of CCR5, CCR2, SDF1, and HLA genes can predict the long-term nonprogressor status in human immunodeficiency virus-1-infected individuals. *Blood* 93:936–41.

Mangin, K., Lipsitch, M., and Ebert, D. 1995. Virulence and transmission models of 2 microsporidia in Daphnia magna. *Parasitology* 111:133–42.

Marsh, K. and Snow, R. W. 1997. Host-parasite interaction and morbidity in malaria endemic areas. *Philos Trans R Soc Lond B Biol Sci* 352:1385–94.

Massad, E. 1987. Transmission rates and the evolution of pathogenesis. *Evolution* 41:1127–30.

May, R. M. and Anderson, R. M. 1983. Epidemiology and genetics in the coevolution of parasites and hosts. *Proc. R. Soc. Lond. B.* 219:281–313.

May, R. M. and Nowak, M. A. 1995. Coinfection and the evolution of parasite virulence. *Proc. R. Soc. Lond. B.* 261:209–215.

McCune, J. 2001. The dynamics of CD4+ T-cell depletion in HIV disease. *Nature* 410:974–9.

Mead-Briggs, A. R. and Vaughan, J. A. 1975. The differential transmissibility of Myxoma virus strains of differing virulence grades by the rabbit flea Spilopsyllus cuniculi (Dale). *J Hyg (Lond)* 75:237–47.

Mellors, J. W., Rinaldo, C. R., J., Gupta, P., White, R. M., Todd, J. A., and Kingsley, L. A. 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 272:1167–70.

Merchant, J., Kerr, P., Simms, N., Hood, G., Pech, R., and Robinson, A. 2003a. Monitoring the spread of myxoma virus in rabbit Oryctolagus cuniculus populations on the southern tablelands of New South Wales, Australia. III. Release, persistence and rate of spread of an identifiable strain of myxoma virus. *Epidemiol Infect* 130:135–47.

Merchant, J., Kerr, P., Simms, N., and Robinson, A. 2003b. Monitoring the spread of myxoma virus in rabbit Oryctolagus cuniculus populations on the southern tablelands of New South Wales, Australia. I. Natural occurrence of myxomatosis. *Epidemiol Infect* 130:113–21.

Messenger, S. L., Molineux, I. J., and Bull, J. J. 1999. Virulence evolution in a virus obeys a trade-off. *Proc R Soc Lond B Biol Sci* 266:397–404.

Metzner, K. J., Jin, X., Lee, F. V., Gettie, A., Bauer, D. E., Di Mascio, M., Perelson, A. S., Marx, P. A., Ho, D. D., Kostrikis, L. G., and Connor, R. I. 2000. Effects of in vivo CD8(+) T cell depletion on virus replication in rhesus macaques immunized with a live, attenuated simian immunodeficiency virus vaccine. *J Exp Med* 191:1921–31.

Mims, C. A., Nash, A., and Stephen, J. 2001. Mims' pathogenesis of infectious disease. Academic Press, London, UK, fifth edition.

Mohri, H., Bonhoeffer, S., Monard, S., Perelson, A., and Ho, D. 1998. Rapid turnover of T lymphocytes in SIV- infected rhesus macaques. *Nature* 279:1223–1227.

Mohri, H., Perelson, A. S., Tung, K., Ribeiro, R. M., Ramratnam, B., Markowitz, M., Kost, R., Hurley, A., Weinberger, L., Cesar, D., Hellerstein, M. K., and Ho, D. D. 2001. Increased turnover of T lymphocytes in HIV-1 infection and its reduction by antiretroviral therapy. *J Exp Med* 194:1277–87.

Monod, J. 1949. The growth of bacterial cultures. *Ann Rev Microb* 3:371–394.

Morand, S., Manning, S. D., and Woolhouse, M. E. 1996. Parasite-host coevolution and geographic patterns of parasite infectivity and host susceptibility. *Proc R Soc Lond B Biol Sci* 263:119–28.

Mosquera, J. and Adler, F. R. 1998. Evolution of virulence: a unified framework for coinfection and superinfection. *J Theor Biol* 195:293–313.

Muller, V., Maree, A. F., and De Boer, R. J. 2001. Release of virus from lymphoid tissue affects human immunodeficiency virus type 1 and hepatitis C virus kinetics in the blood. *J Virol* 75:2597–603.

Murali-Krishna, K. and Ahmed, R. 2000. Cutting edge: naive t cells masquerading as memory cells. *J Immunol* 165:1733–7.

Murali-Krishna, K., Lau, L. L., Sambhara, S., Lemonnier, F., Altman, J., and Ahmed, R. 1999. Persistence of memory CD8 T cells in MHC class I-deficient mice. *Science* 286:1377–1381.

Murphy, B. R. and Webster, R. G. 1996. Orthomyxoviruses, pp. 1397–1446. *In* B. Fields, D. M. Knipe, P. M. Howley, R. M. Chanock, J. L. Melnick, T. P. Monath, B. Roizman, and S. E. Straus (eds.), Field's virology, volume 1, chapter 46. Lippincott-Raven, Philadelphia, PA USA, 3rd edition.

Nelson, P. W., Mittler, J. E., and Perelson, A. S. 2001. Effect of drug efficacy and the eclipse phase of the viral life cycle on estimates of HIV viral dynamic parameters. *J Acquir Immune Defic Syndr* 26:405–12.

Novitsky, V., Gilbert, P., Peter, T., McLane, M. F., Gaolekwe, S., Rybak, N., Thior, I., Ndung'u, T., Marlink, R., Lee, T. H., and Essex, M. 2003. Association between virus-specific t-cell responses and plasma viral load in human immunodeficiency virus type 1 subtype c infection. *J Virol* 77:882–90.

Nowak, M. A., Anderson, R. M., McLean, A. R., Wolfs, T. F., Goudsmit, J., and May, R. M. 1991. Antigenic diversity thresholds and the development of AIDS. *Science* 254:963–9.

Nowak, M. A., Bonhoeffer, S., Hill, A. M., Boehme, R., Thomas, H. C., and McDade, H. 1996. Viral dynamics in hepatitis B virus infection. *Proc Natl Acad Sci U S A* 93:4398–402.

Nowak, M. A. and May, R. M. 1994. Superinfection and the evolution of parasite virulence. *Proc. R. Soc. Lond. B.* 255:81–89.

Ogg, G. S., Jin, X., Bonhoeffer, S., Moss, P., Nowak, M. A., Monard, S., Segal, J. P., Cao, Y., Rowland-Jones, S. L., Hurley, A., Markowitz, M., Ho, D. D., McMichael, A. J., and Nixon, D. F. 1999. Decay kinetics of human immunodeficiency virus-specific effector cytotoxic T lymphocytes after combination antiretroviral therapy. *Journal of Virology* 73:797–800.

Pakker, N., Notermans, D., de Boer, R., Roos, M., de Wolf, F., Hill, A., Leonard, J., Danner, S., Miedema, F., and Schellekens, P. 1998. Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: a composite of redistribution and proliferation. *Nat Med* 4:208–14.

Pedraza, M., del Romero, J., Roldan, F., Garcia, S., Ayerbe, M., Noriega, A. R., and Alcami, J. 1999. Heterosexual transmission of HIV-1 is associated with high plasma viral load levels and a positive viral isolation in the infected partner. *J AIDS* 21:120–125.

Peeters, M., Piot, P., and van der Groen, G. 1991. Variability among HIV and SIV strains of African origin. *AIDS* 5 Suppl 1:S29–36.

Perelson, A., Essunger, P., Cao, Y., Vesanen, M., Hurley, A., Saksela, K., Markowitz, M., and Ho, D. 1997. Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature* 387:188–191.

Perelson, A., Neumann, A., Markowitz, M., Leonard, J., and Ho, D. 1996. HIV-1 dynamics in vivo: virion clearance rate, infected cell lifespan, and viral generation time. *Science* 271:1582–1586.

Perelson, A. S. 2002. Modelling viral and immune system dynamics. *Nature Rev Immunol* 2:28–36.

Peters, C. J., Buchmeier, M., Rollin, P. E., and Ksiazek, T. G. 1996. Arenaviruses, pp. 1521–1552. *In* B. Fields, D. M. Knipe, P. M. Howley, R. M. Chanock, J. L. Melnick, T. P. Monath, B. Roizman, and S. E. Straus (eds.), Field's virology, volume 2, chapter 50. Lippincott-Raven, Philadelphia, PA USA, 3rd edition.

Piatak, M., J., Saag, M. S., Yang, L. C., Clark, S. J., Kappes, J. C., Luk, K. C., Hahn, B. H., Shaw, G. M., and Lifson, J. D. 1993. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science* 259:1749–54.

Pilyugin, S. S. and Antia, R. 2000. Modeling immune responses with handling time. *Bull Math Biol* 62:869–90.

Pirt, S. J. 1975. Principles of microbe and cell cultivation. Blackwell Scientific publications, Oxford.

Ploegh, H. L. 1998. Viral strategies of immune evasion. *Science* 280:248–53.

Poulin, R. and Combes, C. 1999. The concept of virulence: interpretations and implications. *Parasitol Today* 15:474–5.

Poulin, R. and Morand, S. 2000. The diversity of parasites. *Q Rev Biol* 75:277–93.

Quinn, T. C., Wawer, M. J., Sewankambo, N., Serwadda, D., Li, C., Wabwire-Mangen, F., Meehan, M. O., Lutalo, T., and Gray, R. H. 2000. Viral load and heterosexual transmission of Human Immunodeficiency Virus type 1. *N Engl J Med* 342:921–929.

Ramratnam, B., Bonhoeffer, S., Binley, J., Hurley, A., Zhang, L., Mittler, J. E., Markowitz, M., Moore, J. P., Perelson, A. S., and Ho, D. D. 1999. Rapid production and clearance of HIV-1 and hepatitis C virus assessed by large volume plasma apheresis. *Lancet* 354:1782–5.

Rauch, E., Sayama, H., and Bar-Yam, Y. 2003. Dynamics and genealogy of strains in spatially extended host-pathogen models. *J Theor Biol* 221:655–64.

Read, A. 1994. The evolution of virulence. *Trends Microbiol* 2:73–76.

Read, A., Aaby, P., Antia, R., Ebert, D., Ewald, P., Gupta, S., Holmes, E., Sasaki, A., Shields, D., Taddei, F., and E.R., M. 1999. What can evolutionary biology contribute to understanding virulence?, pp. 205–215. *In* Stearns, S. C. (ed.), Evolution in health and disease. Oxford University Press, New York, NY.

Read, A. F., Mackinnon, M. J., Anwar, M. A., and Taylor, L. H. 2002. Kin-selection models as evolutionary explanations of malaria, pp. 165–177. *In* U. Dieckmann, J. A. J. Metz, M. W. Sabelis, and K. Sigmund (eds.), Adaptive dynamics of infectious disease: in pirsuit of virulence management. Oxford University Press.

Read, J. and Keeling, M. 2003. Disease evolution on networks: the role of contact structure. *Proc R Soc Lond B Biol Sci* 270:699–708.

Regoes, R. R., Nowak, M. A., and Bonhoeffer, S. 2000. Evolution of virulence in a heterogeneous host population. *Evolution* 54:64–71.

Revy, P., Sospedra, M., Barbour, B., and Trautmann, A. 2001. Functional antigen-independent synapses formed between T cells and dendritic cells. *Nat Immunol* 2:925–31.

Rey-Cuille, M. A., Berthier, J. L., Bomsel-Demontoy, M. C., Chaduc, Y., Montagnier, L., Hovanessian, A. G., and Chakrabarti, L. A. 1998. Simian immunodeficiency virus replicates to high levels in sooty mangabeys without inducing disease. *J Virol* 72:3872–86.

Rolff, J. and Siva-Jothy, M. 2003. Invertebrate ecological immunology. *Science* 301:472–5.

Sabelis, M. W. and Metz, J. A. J. 2002. Taking stock: relating theory to experiment, pp. 379–398. *In* U. Dieckmann, J. A. J. Metz, M. W. Sabelis, and K. Sigmund (eds.), Adaptive dynamics of infectious disease: in pirsuit of virulence management. Oxford University Press.

Sacristan, S., Malpica, J., Fraile, A., and Garcia-Arenal, F. 2003. Estimation of population bottlenecks during systemic movement of tobacco mosaic virus in tobacco plants. *J Virol* 77:9906–11.

Sasaki, A. and Iwasa, Y. 1991. Optimal growth schedule of pathogens within a host: switching between lytic and latent cycles. *Theor Popul Biol* 39:201–39.

Schall, J. J. 2002. Parasite virulence, pp. 283–313. *In* E. E. Lewis, J. F. Campbell, and M. V. K. Suchedeo (eds.), The behavioural evology of parasites. CAB International.

Schulman, J. L. 1967. Experimental transmission of influenza virus infection in mice. IV. Relationship of transmissibility of different strains of virus and recovery of airborne virus in the environment of infector mice. *J Exp Med* 125:479–88.

Schulman, J. L. 1970. Effects of immunity on transmission of influenza: experimental studies. *Prog Med Virol* 12:128–60.

Segal, S. and Hill, A. 2003. Genetic susceptibility to infectious disease. *Trends Microbiol* 11:445–8.

Sharpe, F. R. and Lotka, A. J. 1911. A problem in age-distribution. *Phil Mag* 21:435–438.

Slifka, M. K. and Ahmed, R. 1998. Long-lived plasma cells: a mechanism for maintaining persistent antibody production. *Curr Opin Immunol* 10:252–8.

Smith, J. A. and Martin, L. 1973. Do cells cycle? *Proc Natl Acad Sci USA* 85:411–426.

Smith, T. 2002. Imperfect vaccines and imperfect models. *Trends Ecol Evol* 17:154–156.

Soubeyrand, B. and Plotkin, S. A. 2002. Microbial evolution (Communication arising): Antitoxin vaccines and pathogen virulence. *Nature* 417:609–10.

Staprans, S. I., Dailey, P. J., Rosenthal, A., Horton, C., Grant, R. M., Lerche, N., and Feinberg, M. B. 1999. Simian immunodeficiency virus disease course is predicted by the extent of virus replication during primary infection. *J Virol* 73:4829–39.

Sun, Y., Iglesias, E., Samri, A., Kamkamidze, G., Decoville, T., Carcelain, G., and Autran, B. 2003. A systematic comparison of methods to measure HIV-1 specific CD8 T cells. *J Immunol Methods* 272:23–34.

Taha, M., Deghmane, A., Antignac, A., Zarantonelli, M., Larribe, M., and Alonso, J. 2002. The duality of virulence and transmissibility in Neisseria meningitidis. *Trends Microbiol* 10:376–82.

Taranger, J., Trollfors, B., Bergfors, E., Knutsson, N., Sundh, V., Lagergard, T., Lind-Brandberg, L., Zackrisson, G., White, J., Cicirello, H., Fusco, J., and Robbins, J. 2001. Mass vaccination of children with pertussis toxoid–decreased incidence in both vaccinated and nonvaccinated persons. *Clin Infect Dis* 33:1004–10.

Taylor, L. H. and Read, A. F. 1997. Why so few tranmission stages? reproductive restraint by malaria parasites. *Parasit Today* 13:135–140.

Taylor, L. H. and Read, A. F. 1998. Determinants of transmission success of individual clones from mixed- clone infections of the rodent malaria parasite, Plasmodium chabaudi. *Int J Parasitol* 28:719–25.

Theodorou, I., Capoulade, C., Combadiere, C., and Debre, P. 2003. Genetic control of HIV disease. *Trends Microbiol* 11:392–7.

Thrall, P. and Burdon, J. 2003. Evolution of virulence in a plant host-pathogen metapopulation. *Science* 299:1735–7.

Tortora, G. J., Funke, B. R., and Case, C. L. 1992. MIcrobiology: an introduction. The Benjamin/Cummings Publishing company, Inc., Redwood city, California, USA, forth edition.

Traub, E. 1936. The epidemiology of lymphocitic choriomeningitis in white mice. *J Exp Med* 64:183–200.

Traub, E. 1939. Epidemiology of LCM in a mouse stock observed for four years. *J Exp Med* 70:801–817.

Turner, P., Cooper, V., and Lenski, R. 1998. Tradeoff between horizontal and vertical modes of transmission in bacterial plasmids. *Evolution* 52:315–29.

Turner, P. E. and Chao, L. 1999. Prisoner's dilemma in an RNA virus. *Nature* 398:441–3.

Turner, P. E. and Elena, S. F. 2000. Cost of host radiation in an RNA virus. *Genetics* 156:1465–70.

van Baalen, M. 1998. Coevolution of recovery ability and virulence. *Proc R Soc Lond B Biol Sci* 265:317–25.

van Baalen, M. 2002. Contact networks and the evolution of virulence, pp. 85–103. *In* U. Dieckmann, J. A. J. Metz, M. W. Sabelis, and K. Sigmund (eds.), Adaptive dynamics of infectious disease: in pirsuit of virulence management. Oxford University Press.

van Baalen, M. and Sabelis, M. 1995. The dynamics of multipe infection and the evolution of virulence. *Am. Nat.* 146:881–910.

van Stipdonk, M. J., Lemmens, E. E., and Schoenberger, S. P. 2001. Naive CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. *Nat Immunol* 2:423–9.

Veiga-Fernandes, H., Walter, U., Bourgeois, C., McLean, A., and Rocha, B. 2000. Response of naive and memory CD8+ T cells to antigen stimulation in vivo. *Nat Immunol* 1:47–53.

von Foerster, H. 1959. Some remarks on changing populations, pp. 328–407. *In* F. Stohlman Jr. (ed.), The kinetics of cellular proliferation. Grune and Stratton, New York.

Wei, X., Ghosh, S., Taylor, M., Johnson, V., Emini, E., Deutsch, P., Lifson, J., Bonhoeffer, S., Nowak, M., Hahn, B., Saag, M., and Shaw, G. 1995. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 373:117–122.

Weiss, R. A. 2002. Virulence and pathogenesis. *Trends Microbiol* 10:314–7.

Weston, S. A. and Parish, C. R. 1990. New fluorescent dyes for lymphocyte migration studies. analysis by flow cytometry and fluorescence microscopy. *J Immun Meth* 133:87–97.

Williams, P. D. and Day, T. 2001. Interactions between sources of mortality and the evolution of parasite virulence. *Proc R Soc Lond B Biol Sci* 268:2331–7.

Windsor, D. 1998. Most of the species on Earth are parasites. *Int J Parasitol* 28:1939–41.

Wodarz, D., Hall, S. E., Usuku, K., Osame, M., Ogg, G. S., McMichael, A. J., Nowak, M. A., and Bangham, C. R. 2001. Cytotoxic T-cell abundance and virus load in human immunodeficiency virus type 1 and human T-cell leukaemia virus type 1. *Proc R Soc Lond B Biol Sci* 268:1215–21.

Wodarz, D. and Krakauer, D. C. 2000. Defining CTL-induced pathology: implications for HIV. *Virology* 274:94–104.

Woolhouse, M. E., Dye, C., Etard, J. F., Smith, T., Charlwood, J. D., Garnett, G. P., Hagan, P., Hii, J. L., Ndhlovu, P. D., Quinnell, R. J., Watts, C. H., Chandiwana, S. K., and Anderson, R. M. 1997. Heterogeneities in the transmission of infectious agents: implications for the design of control programs. *Proc Natl Acad Sci U S A* 94:338–42.

Woolhouse, M. E., Taylor, L. H., and Haydon, D. T. 2001. Population biology of multihost pathogens. *Science* 292:1109–12.

Yang, O. O., Kalams, S. A., Rosenzweig, M., Trocha, A., Jones, N., Koziel, M., Walker, B. D., and Johnson, R. P. 1996. Efficient lysis of human immunodeficiency virus type 1-infected cells by cytotoxic T lymphocytes. *J Virol* 70:5799–806.

Yang, O. O., Kalams, S. A., Trocha, A., Cao, H., Luster, A., Johnson, R. P., and Walker, B. D. 1997. Suppression of human immunodeficiency virus type 1 replication by CD8+ cells: evidence for HLA class I-restricted triggering of cytolytic and noncytolytic mechanisms. *J Virol* 71:3120–8.

Zhang, L., Dailey, P. J., He, T., Gettie, A., Bonhoeffer, S., Perelson, A. S., and Ho, D. D. 1999. Rapid clearance of simian immunodeficiency virus particles from plasma of rhesus macaques. *J Virol* 73:855–60.

Zhong, S. and Dobson, C. 1996. Heligmosomoides polygyrus: resistance in inbred, outbred, and selected mice. *Exp Parasitol* 82:122–31.